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THE EFFECT OF DIFFERENT FERTILITY LEVELS ON YIELDS OF INTENSIVELY MANAGED COFFEE IN PUERTO RICO
Fernando Abruña, José Vicente, and Servando Silva 141

PARATHION-RESIDUE STUDIES ON ACEROLA ..... *Julián Roldán* 147

INFLUENCE OF ROOT-INDUCING SUBSTANCES AND TIME INTERVALS ON THE ROOTING OF ACEROLA CUTTINGS
Raúl Abrams and George C. Jackson 152

INFLUENCE OF THE AGE OF SUGARCANE ON ITS LEAF-NUTRIENT (N-P-K) CONTENT ..... *George Samuels* 159

EVIDENCE OF THE PRESENCE OF THE TOBACCO-ETCH VIRUS IN PUERTO RICO ..... *L. López Matos* 171

PHOSPHATE RETENTION BY MUCK SOILS ..... *Julián Roldán* 182

FACTORS THAT GOVERN THE SENSITIVITY OF OAT SEED TO X-RAYS
C. L. González-Molina 194

RESEARCH NOTE
Vitamin C in Acerola and Rose Hip ..... *C. F. Asenjo* 212

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Vol. XLIII July 1959 No. 3

The Effect of Different Fertility Levels on Yields of Intensively Managed Coffee in Puerto Rico[1]

Fernando Abruña, José Vicente-Chandler, and Servando Silva[2]

INTRODUCTION

Coffee plantations in Puerto Rico generally consist of old, unselected coffee trees growing in very dense shade provided by old shade trees. Insects and diseases are not controlled, the coffee trees are rarely pruned, and little fertilizer is applied. It is therefore not surprising that yields average only about 150 pounds of market coffee per acre, probably the lowest of any coffee-producing country.

In 1951, the authors started experimenting with a system for the intentensive management of coffee in Puerto Rico. The trees were planted close together in rows with no shade, in line with Cowgill's (*1*)[3] work in Guatemala. The high-yielding Bourbon variety was used with periodic spraying for pest control, heavy fertilization, systematic pruning, and proper soil- and water-conserving practices. These plantings produced very high yields—the oldest has produced four crops averaging almost 2,000 pounds of market coffee per acre, yearly. It immediately became apparent that information on the fertility requirements of such intensively managed, high-yielding plantings was urgently needed.

Information on the fertilizer requirements of coffee in Puerto Rico is limited. McClelland (*3*) found that shaded coffee growing on a soil not typical of the Coffee Region responded in yield to applications of potash, particularly when nitrogen was also used, but did not respond to phos-

[1] This work was carried out cooperatively between the U. S. Department of Agriculture and the Agricultural Experiment Station of the University of Puerto Rico, Río Piedras, P.R.

[2] Soil Scientist, Project Supervisor, and Physical Science Aid, respectively, Soil and Water Conservation Research Division, Agricultural Research Service, U. S. Department of Agriculture.

Deep appreciation is expressed to Saulo J. Rodríguez, Assistant Agronomist in Charge of the Castañer Substation, and to Jorge Toro, former Assistant in Horticulture of the Agricultural Experiment Station of the University of Puerto Rico, for their wholehearted cooperation in helping to carry out this experiment.

[3] Italic numbers in parentheses refer to Literature Cited p. 146.

phorus applications. On the other hand Gómez, *et al.* (*2*), found that shaded trees growing on a typical soil (Catalina) responded to phosphorus applications and to a lesser extent to nitrogen, but did not respond to potash. There is no information available in Puerto Rico on the fertility requirements of coffee intensively managed as described above.

MATERIALS AND METHODS

The experiment was carried out at Castañer at an elevation of about 2,000 feet, mean monthly temperatures varying from about 70° to 80° F., and about 70 inches of annual rainfall. A severe drouth occurred in 1957, with rainfall that year of less than 50 inches.

The soil was steep (45-percent slope), eroded, Alonso clay, the upper 6 inches of which had about 3.5-percent organic matter with a pH of 4.8, an exchange capacity of 22 m.e., and 12 m.e. of exchangeable bases per 100 gm. of soil. The soil is well drained and has excellent physical condition.

Arabica coffee of the Bourbon variety was planted 3 feet apart in two rows 4 feet apart, forming hedges which are 10 feet apart, with about 2,000 trees per acre. Grass was allowed to grow between hedges to control erosion, but the ground under the trees was kept free from weeds. No stock was used. The seedlings were transplanted to the field when about 2½ feet tall, with a ball of earth surrounding the roots. The trees were encouraged to produce three to four vertical stems and old, diseased, or weak branches were removed once a year. The plantings were sprayed four times yearly with a mixture containing Parathion, Dieldrin, and Copper A, to help control insects and diseases. Two tons of limestone and 400 pounds of magnesium sulfate were applied per acre to all plots every 2 years. All plots received two light applications of nitrogen over the first 4 months, after which the specific differential treatments were initiated. Since up to the present no clear evidences of minor element deficiencies had been noted these have not been applied.

A randomized block design was used with all treatments replicated 16 times. Individual plots consisted of 12 trees, 6 trees in each of the 2 rows of a hedge, and surrounded by ditches to reduce the possibility of fertilizer washing into adjoining plots. Seven replications were planted in July 1954 and the other nine in July 1955.

The eight fertilizer treatments are listed in the first three columns of table 1. Zero, 150, and 300 pounds per acre yearly each of nitrogen (from ammonium sulfate), phosphoric acid (from 20-percent superphosphate), and potash (from potassium chloride) were tested in the presence of the highest level of the other two nutrients. In addition, a no-fertilizer treatment was included. Fertilizer was applied in three equal applications in April, July, and October of each year.

RESULTS AND DISCUSSION

The results obtained in the first three crops, starting 2½ years after planting, are summarized in table 1 and figure 1. Yields for the first two crops were more than doubled, and those of 1958 more than tripled by proper fertilization, when compared with no fertilization.

Yields of market coffee were increased by 8.7, 3.7, and 7.3 hundredweights per acre in 1956, 1957, and 1958, respectively, by the application of 150 pounds of nitrogen per acre yearly (fig. 2A). During the very dry year of 1957 there was no evidence of a response beyond this level.

In 1956, yields were increased by 5.7 hundredweights per acre by the application of 150 pounds of potash per acre yearly. In 1957, yields were increased by 4 hundredweights per acre, by the application of 300 pounds of potash. In 1958, yields were increased by 4 hundredweights per acre by the application of 150 pounds of potash, and by an additional 3.8 hundredweights by the application of 300 pounds of potash per acre yearly (fig. 2B).

TABLE 1.—*The effect of 3 levels each of nitrogen, phosphorus, and potassium on yields of intensively managed coffee grown in Alonso clay at Castañer, P. R.*

Treatment[1]			Yields of market coffee		
N	P_2O_5	K_2O	1956[2]	1957	1958
Lb./A.	*Lb./A.*	*Lb./A.*	*Cwt./A.*	*Cwt./A.*	*Cwt./A.*
0	0	0	9.2	6.6	5.6
300	300	300	18.9	14.0	18.9
0	300	300	7.6	10.5	9.3
150	300	300	16.3	14.2	16.6
300	300	0	13.7	10.0	11.1
300	300	150	19.4	13.1	15.1
300	0	300	13.9	15.3	17.4
300	150	300	18.1	15.9	17.4
L.S.D. 5-percent level			5.7	3.6	3.6
1-percent level			7.7	4.8	4.8

[1] In 3 equal applications yearly.

[2] 7 replications this year—16 in successive years.

There was no significant response to phosphorus applications during any year, the apparent response during the first year not attaining statistical significance.

The data indicated the need for at least 150 pounds each of nitrogen and potash per acre yearly. With favorable weather somewhat more nitrogen may be required. With increasing growth of trees, applications of at least 300 pounds of potash per acre were required for maximum yields with the third crop. At least in young plantations, applications of phosphorus seem

desirable. Under some conditions, heavier applications of fertilizer than indicated above may be warranted. There can be no doubt that heavy fertilization of intensively managed coffee is very profitable considering the magnitude of the yield increases obtained and the relative prices of coffee and fertilizer.

Analysis of leaf samples taken from all plots when the 1958 crop was

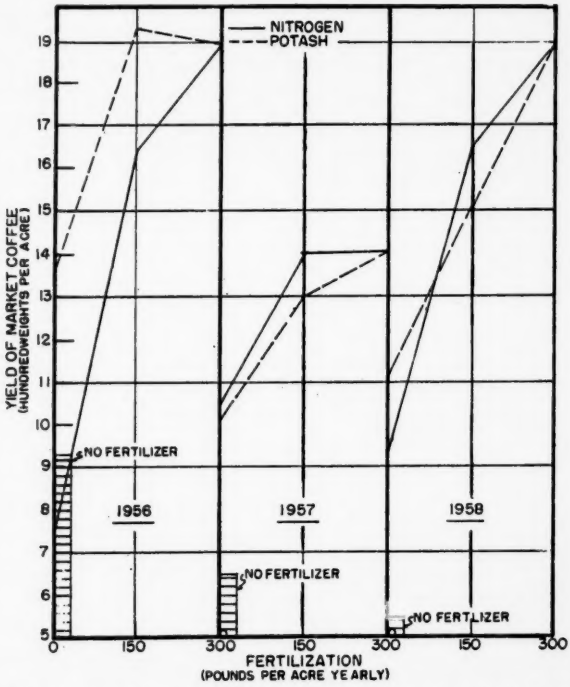

FIG. 1.—The effect of 3 levels each of nitrogen and potash on yields of intensively managed coffee, Puerto Rico, 1956–58.

maturing showed that nitrogen content of the leaves increased with nitrogen rates, averaging 2.3, 2.8, and 3.0 percent at the 0-, 150-, and 300-pound levels, respectively. Similarly, potassium content averaged 0.98, 1.67, and 2.18 percent at the 0-, 150-, and 300-pound levels of potash, respectively. Neither the phosphorus content, which ranged from 0.15 to 0.19 percent, nor the calcium content, which ranged from 0.90 to 1.2 percent, was appreciably affected by the treatments, although highest leaf contents of these nutrients occurred when no nitrogen was applied. It is of interest to note that the magnesium content of the leaves dropped from 0.85 to 0.59 and 0.45 percent, respectively, with each increase in potash level.

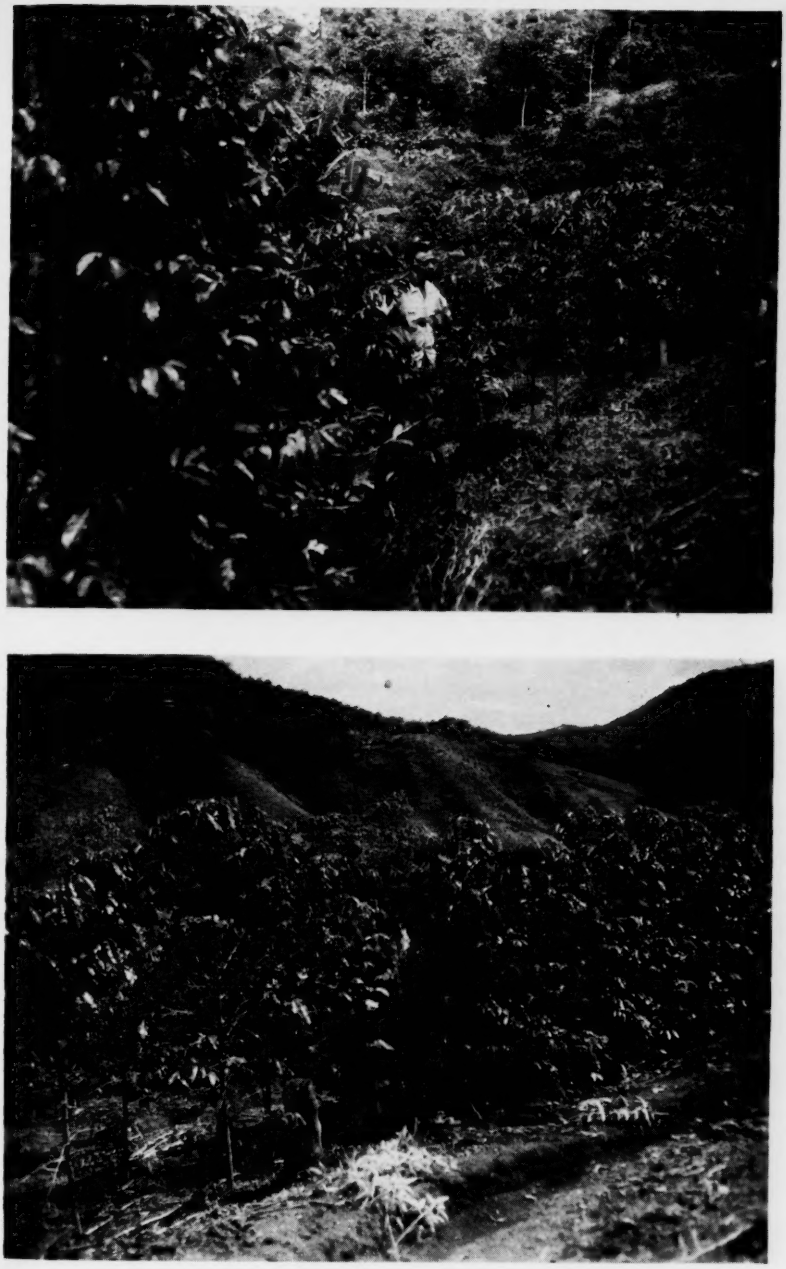

FIG. 2.—Intensively managed coffee responds strongly to fertilization with nitrogen and potash. In both cases trees received an abundance of nutrients other than those indicated: A (top), Trees on right received no nitrogen, those on left 300 pounds per acre yearly; B (bottom), trees on left received no potash, those on right 300 pounds per acre yearly.

SUMMARY

The effect of three levels each of nitrogen, phosphoric acid, and potash, on yields of intensively managed coffee grown in Alonso clay at Castañer were determined. Yields averaging in excess of 15 hundredweights of market coffee per acre were obtained over 3 successive years, compared to an Islandwide average of about 150 pounds.

A marked response to the application of 150 pounds of nitrogen per acre occurred during all 3 years, with a strong indication of a response to an additional 150 pounds of nitrogen during the 2 years of normal rainfall. A strong response to the application of 150 pounds of potash per acre occurred during the first year, a response to the 300-pound application the second year, and to the 150- and 300-pound levels the third year. There was no significant response to phosphorus in any year.

RESUMEN

Se determinó el efecto de tres niveles de nitrógeno, ácido fosfórico y potasa en el rendimiento de café cultivado intensivamente en suelo Alonso arcilloso, en Castañer. El rendimiento promedio fué de más de 15 quintales de café pilado por cuerda durante 3 años consecutivos, lo cual compara favorablemente con el promedio de 150 libras por cuerda para la Isla en general.

Hubo una reacción favorable en rendimiento a la aplicación de 150 libras de nitrógeno por cuerda durante todos los 3 años. Durante los 2 años de lluvia abundante hubo indicación fuerte de una reacción a la aplicación de 150 libras de nitrógeno adicionales. También hubo una reacción favorable en rendimiento a la aplicación de 150 libras de potasa por cuerda el primer año, y a la aplicación de 300 libras por cuerda el segundo año. Durante el tercer año hubo una reacción favorable a la aplicación de 150 y de 300 libras de potasa por cuerda. La aplicación de fósforo no afectó significativamente los rendimientos de café.

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Parathion-Residue Studies on Acerola

Julián Roldán[1]

INTRODUCTION

The economic value of using chemicals for controlling pests so as to assure good crop production has not yet been determined in Puerto Rico. Nevertheless farmers are continually searching for new chemicals for pest control. New chemicals which are far better than those used in years past are continually introduced, and with their introduction new problems have arisen, such as disposal of persisting poisonous residues. Besides making food unfit for human consumption, the indirect effect of the residues in producing off-flavors makes the problem still more difficult to solve.

RESIDUAL ACTION OF PESTICIDES

After plants are sprayed with an insecticide, this initial chemical deposit ceases to be such as soon as air, light, rain, or weathering affect it (*5*)[2]. Once the deposit has been made, part of it sloughs off and is wasted. Some adheres to the plant parts and remains there, but some may penetrate into the plant. The insecticide that enters the plant parts is not easily recognizable after it is released by the plant. Thus, to determine the residual action of an insecticide there is need to study its vapor pressure, sticking power, solubility, and absorption into the surface on which it is applied, as well as resistance to chemical change (*4*).

The ideal insecticide should have enough residual action to protect the crop, but should also decompose to inert material as soon as the need for protection ends.

MODE OF ACTION AND CHEMISTRY OF PARATHION

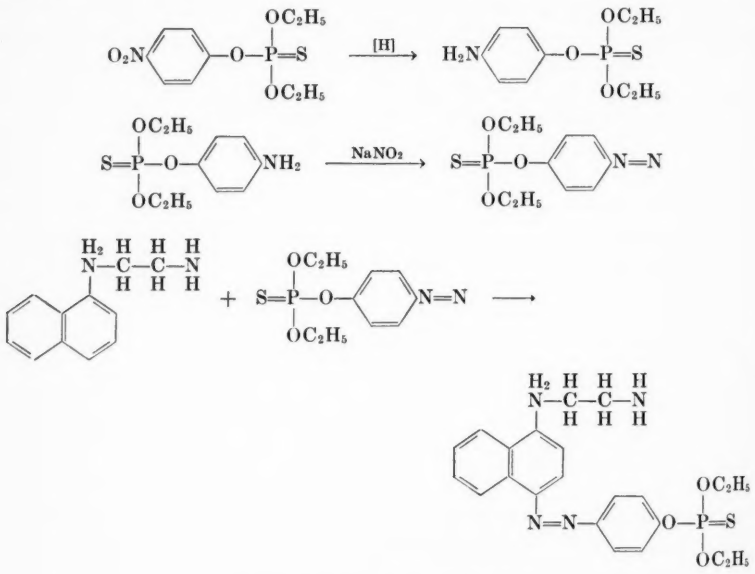
Parathion (O,O-diethyl-O-p-nitrophenyl thiophosphate) is a high-boiling deep-brown to yellow liquid with a specific gravity of 1.26. It is slightly soluble in water and in petroleum ether, but is completely miscible with acetone, ethyl ether, alcohols, esters, and animal or vegetable oils. It is hydrolyzed in the presence of alkaline materials such as lime, lime-sulfur, and bordeaux mixture. Parathion is prepared by having phosphorous thiotrichloride react with sodium ethoxide and the product with sodium paranitrophenate. The high toxicity of Parathion is associated with the presence of the $(C_2H_5O)_2P$-group (*6*, *8*).

[1] Assistant Chemist, Agricultural Experiment Station, University of Puerto Rico, Río Piedras, P.R. The author wishes to express his sincere gratitude to Mario Pérez Escolar, Assistant Entomologist, for his valuable help during this investigation.

[2] Italic numbers in parentheses refer to Literature Cited, p. 151.

The chemical acetylcholine, acetylethanol trimethyl ammonium hydroxide, is a derivative of choline and is regarded as important in the mechanism of nerve action. It is believed to be produced in the nerve fibers and to help in the transmission of nerve impulses (*3*, *7*). The enzyme cholinesterase hydrolyzes acetylcholine into inactive choline and acetic acid, thus preventing body accumulation of acetylcholine. If Parathion is present it will poison the enzyme cholinesterase and the acetylcholine will accumulate, thus causing uncoordinated nervous activity throughout an entire animal, and resulting in its death.

In the determination of Parathion use is made of a method developed by Bratton and Marshall (*2*) for the determination of sulfanilamide in blood and urine. In this method the sulfanilamide is diazotized and then coupled with N (1 naphtyl) ethylenediamine dihydrochloride. Averell and Norris (*1*) reduced the nitro group to an amino group in an acid medium. The amino group is then diazotized with sodium nitrite and then coupled with N (1 naphtyl) ethylenediamine dihydrochloride. The reaction in steps is:



MATERIALS AND METHODS

A 5-year-old grove of B. 17 variety of West Indian cherry was selected for an experiment at the Isabela Substation. The design of the experiment was a balanced incomplete block with four treatments replicated six times.

Each replicate included four trees. Buffer rows were left between plots to reduce contamination during spray applications. The treatments given for the first experiment were as follows:

1. 3 sprays divided as follows:
   A. 1 spray at blossom time
   B. A second spray 10 days after
   C. A third spray 10 days after the second
2. 1 spray at blossom time
3. 2 sprays divided as follows:
   A. 1 spray 10 days after blossom time
   B. A second spray 10 days after the first
4. Untreated check

Parathion was applied as a wettable powder at the rate of 1.5 pounds of the 15-percent formulation in 100 gallons of water. Each tree received 2.6 gallons of the spray per application with a power-motor sprayer. To prevent drifting as much as possible the trees were sprayed starting early in the morning using a canvas to protect one plot from the other.

Although precautions were taken to avoid drifting caused by the wind, it was never possible to obtain complete control.

The spray applied per tree can be estimated only approximately, for there was no control of the rate of flow of the Parathion solution or of the time factor. Another factor was that the man handling the sprayer never moved at a constant speed while spraying.

SAMPLING

Precautions were taken while the sampling was done, for contamination was easy. Therefore, those doing the sampling were required to wash their hands once they had finished sampling the corresponding plot. Four men did the sampling to assure samples as representative as possible. Two of the men went around the tree clockwise while the other two went counter-clockwise. Care was taken that they took fruit from all parts of the trees. Fruits that were too ripe were not picked, for West Indian cherries are very perishable. If the fruits selected were too ripe they were dripping juice by the time they reached the laboratory. This was avoided so that the analyses were not spoiled.

EXTRACTION TECHNIQUES

Five hundred grams of whole cherries were weighed and mixed with 500 ml. of the extracting solvent, benzene. Both cherries and solvent were placed in a steel container $7\frac{1}{8}''$ wide and $8\frac{1}{2}''$ long. The mouth of the steel container was covered with plastic cloth, the commercial name of which was "Clear Shelene", and the containers were placed in a shaking-machine

and shaken end-to-end at a speed of 8.5 revolutions per minute during 1 hour. Aliquots were taken from the filtered extracted solution and analyzed for Parathion. This method of extraction was discarded because it did not accomplish the objective of the experiment.

A second extraction method was tried. In this the cherries were ground in a Waring Blendor. Two hundred and fifty grams of the pulp were mixed with 200 gm. of benzene, and the mixture was placed in a separatory funnel. The mixture was shaken occasionally for 30 minutes and an emulsion was formed. Because of this it was impossible to separate the pulp from the extracting solvent, so the method was discarded.

In a third extraction method the cherries were ground in a Waring Blendor. Two hundred and fifty grams of the pulp were placed in a stainless steel container. To this were added 200 gm. of benzene and the mixture was shaken end to end for 1 hour in a shaking-machine. The benzene extract

TABLE 1.—*Mean content of Parathion in pulp of West Indian cherries picked after spraying 1 to 3 times*

Treatment	Parathion	Time after last application
	(P.p.m.)	*Days*
3 sprays	0.093	4
1 spray	—	20
2 sprays	.050	4

remained as a supernatant liquid and the pulp was easily decanted. This proved to be the best extraction technique.

RESULTS

On September 3, 1957, samples of whole cherries were taken before they were sprayed for a third time. The results of the analyses indicated that there was no Parathion present on the cherries 11 days and 21 days after they had been sprayed.

The experiment was repeated on October 18, 1957. Spray was applied on that date and two additional sprays on October 29 and November 4, respectively. Fruit samples were taken for analysis on November 7. Samples were ground in a Waring Blendor and the pulp was extracted with benzene. The extracts analyzed showed a very low content of Parathion as shown in table 1.

SUMMARY

This paper reports the results obtained in studies carried out with spray deposits left by the insecticide Parathion on West Indian cherries. Although data are not final, it seems that Parathion applied at the rate of 1.5 pounds

per 100 gallons of water per acre to West Indian cherries leaves a residue which is below the limit established for fruits by the Food and Drug Administration of the U. S. Department of Health, Education, and Welfare.

The results thus far obtained indicate that Parathion is rapidly degraded under the conditions of these experiments. It seems too early to make any final recommendations. More experiments have to be performed, especially during other seasons of the year, so that results can be compared and critically evaluated.

RESUMEN

Se presentan los resultados obtenidos en estudios hechos con el insecticida Paración. Los resultados demuestran que el Paración aplicado a árboles de acerola, a razón de 1.5 libras en cien galones de agua por acre, dejan un residuo por debajo de los límites establecidos por el Negociado de Alimentos y Drogas del Departamento de Agricultura Federal.

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Influence of Root-Inducing Substances and Time Intervals on the Rooting of Acerola Cuttings

Raúl Abrams and George C. Jackson[1]

INTRODUCTION

Since the report of the high ascorbic acid content of the juice of its fruit (*1*)[2], the acerola (*Malpighia punicifolia* L.), has become a potential economic crop for Puerto Rico. Beginning with fieldwork at the Agricultural Experiment Station, plantings of acerola have been expanding (*2*) and, at present, a processing plant is operating on the Island and the juice is exported to world markets.

The propagation of hardwood cuttings of acerola is still comparatively recent and no information is available on the root development and propagation by the use of root-inducing substances on cuttings. Since acerola has great economic potentialities for Puerto Rico it was felt that information was needed concerning its propagation by using root-inducing substances. The work performed covers not only the development of roots by the use of hormones, but also the time interval required for maximum root development.

MATERIALS AND METHODS

The study on the rooting of acerola cuttings was started on May 17, 1957, and completed on July 16, 1957. Ten-inch-long hardwood terminal cuttings were taken from healthy 6-year-old acerola trees of clone B. 15. The cuttings were trimmed to 8-inch lengths and the leaf areas were kept at a maximum for, under these conditions, better rooting responses have been reported on other plants (*3*). The cuttings were treated with commercial preparations of indolebutyric and naphthaleneacetic acids which have been reported effective in the rooting of cuttings (*4*).

The treatment consisted of moistening the basal ends of the cuttings and immersing them in the appropriate hormone powder to a depth of one-half inch. The excess powder was removed by tapping the cuttings gently.

The experimental design was a split-plot one in which the two hormone treatments and the control were studied in the whole plots. A subplot consisted of 100 cuttings set 1 inch deep in holes previously made in fumi-

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[2] Italic numbers in parentheses refer to Literature Cited, p. 158.

gated sand beds. The spacing between cuttings was 2 inches both ways. The cuttings were dug from the sand beds at 6-, 8-, and 10-week intervals and rooting percentages and root counts were recorded for each treatment. All treatments were replicated four times.

The cuttings received 80-percent natural light and, through the use of electrically controlled humidifying equipment, they also received 5 seconds of overhead mist spray per minute during daylight hours.

EXPERIMENTAL RESULTS AND DISCUSSION

EFFECT OF ROOT-INDUCING SUBSTANCES ON THE ROOTING OF CUTTINGS

Data on the mean rooting percentages of acerola cuttings treated with root-inducing substances and left in the sand beds during 6, 8, and 10 weeks are presented in table 1 and figure 1.

The standard errors and least differences for significance between means are shown in the following tabulation:

Items	*5 percent*	*1 percent*
Hormone treatments		
Difference between highest and lowest means	12.4	18.1
Difference between 2 adjacents	9.9	15.0
Standard error 2.86 with 6 d.f.		
Time periods		
Highest and lowest	7.4	9.6
Difference between 2 adjacents	6.1	8.3
Standard error 2.05 with 18 d.f.		
Between time intervals for same hormone treatment		
Highest and lowest	12.8	16.7
Difference between 2 adjacents	10.5	14.4
Standard error 3.54 with 18 d.f.		
Between any 2 hormones for same time interval		
Highest and lowest	16.2	22.4
Difference between 2 adjacents	13.1	18.9
Standard error 4.07		

Table 1 shows that, when the cuttings were treated with indolebutyric acid, the response in the number of cuttings that rooted was significant at the 5-percent level. The response of acerola cuttings to the root-inducing substances is presented in a graphic form in figure 1, the uppermost line represents indolebutyric acid and the lowest one the control, indicating a definite response of the cuttings to the use of indolebutyric acid. However, the average difference in mean rooting percentage was not significant between the two hormone treatments.

TABLE 1.—*Mean rooting percentages of acerola cuttings of B. 15 clone treated with root-inducing substances and left in the sand beds for 6, 8, and 10 weeks*

Treatments	Results after number of weeks indicated			Mean
	6	8	10	
Indolebutyric acid	39.3	68.5	70.0	59.3
Naphthaleneacetic acid	39.3	62.0	65.5	55.6
Control	29.3	55.8	54.8	46.6
Mean	36.0	62.1	63.4	53.8

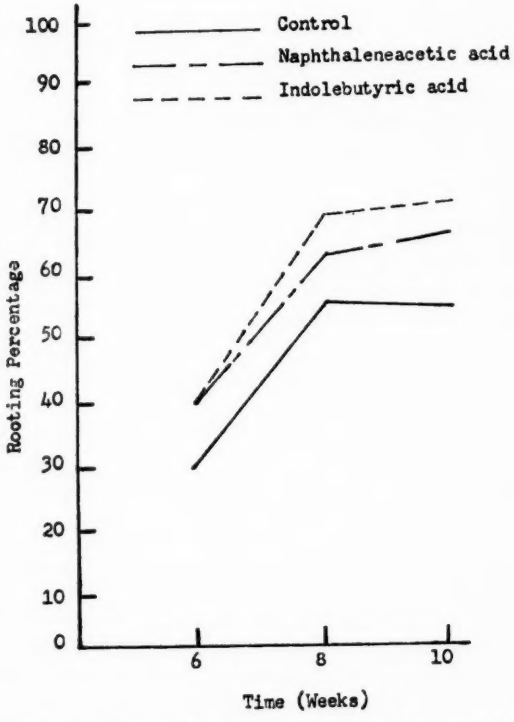

FIG. 1.—Average rooting percentage of acerola cuttings treated with root-inducing substances and left in the sand beds for 6, 8, and 10 weeks.

EFFECT OF DIFFERENT TIME INTERVALS

The results of different time intervals are also presented in table 1 and figure 1. Leaving the cuttings in the sand beds for different time intervals definitely influenced the rooting percentages of the cuttings. The number of cuttings that rooted after 8 and 10 weeks in the sand beds showed significant differences at the 1-percent level over the 6-week period. However, there was no significant difference between the 8- and 10-week intervals, suggesting that of the three intervals studied, the 8-week one is the most appropriate to keep the cuttings in the sand beds.

This result is of importance because a reduction of 2 weeks in the time that the cuttings are to remain in the sand beds represents a more rapid propagation of the acerola and, at the same time, a larger number of cuttings can be propagated during a particular period of time. The mean rooting percentages for the 6-, 8-, and 10-week intervals were 36, 62.1, and 63.4, respectively. This is represented graphically in figure 1, the two uppermost lines representing indolebutyric and naphthaleneacetic acid, respectively.

Table 1 also indicates that, for any combination of time and hormone treatment, the percentage of cuttings that rooted was significant at the 1-percent level when they were left in the sand beds for 8 and 10 weeks as compared to the 6-week time interval. The cuttings treated with indolebutyric acid had rooting percentages 6.5 percent higher after 8 weeks and 4.5 percent higher after 10 weeks than those treated with naphthaleneacetic acid.

According to the comparisons in table 1, there were significant differences in the rooting percentages when the cuttings were treated with hormones at a given time interval. However, these data do not suggest that treating the cuttings with hormones will not cause an increase in the number of cuttings that root, because the contrary is definitely shown in table 1 and figure 1.

AVERAGE NUMBER OF ROOTS PER CUTTING

The results on the average number of roots per cutting are presented in table 2 and figure 2. Treating the acerola cuttings with root-inducing substances definitely increased the number of roots per cutting. The response in the number of roots per cutting when treated with indolebutyric acid was significant at the 1-percent level over naphthaleneacetic acid and the control.

Leaving the cuttings in the sand beds for 8 weeks also caused an increase in the number of roots produced, as indicated in table 2. These data are very similar to those obtained on the rooting percentages of the cuttings. However, when indolebutyric acid was used, leaving the cuttings in the

TABLE 2.—*Mean number of roots per cutting of acerola clone B. 15 when treated with root-inducing substances and left in the sand beds for 6, 8, and 10 weeks*

Treatments	Results after number of weeks indicated			Mean
	6	8	10	
Indolebutyric acid	3.9	4.4	4.4	4.2
Naphthaleneacetic acid	3.1	3.3	3.9	3.4
Control	1.9	2.5	2.6	2.3
Mean	3.0	3.4	3.6	3.3

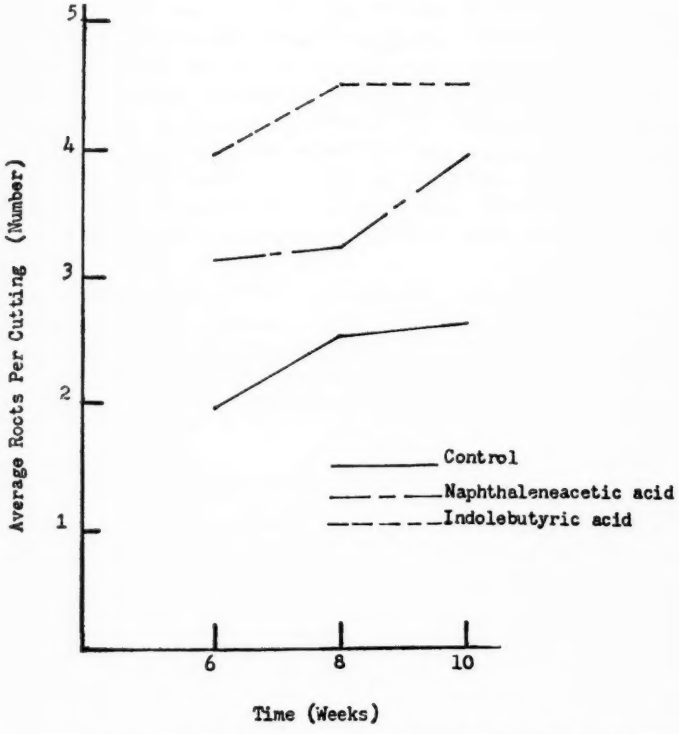

FIG. 2.—Average number of roots per cutting of acerola clone B. 15 when treated with root-inducing substances and left in the sand beds for 6, 8, and 10 weeks.

sand beds from 8 to 10 weeks did not increase the number of roots, while a slight increase was observed with naphthaleneacetic acid and the control. There were highly significant differences in the number of roots per cutting when these were treated with indolebutyric acid and left in the sand beds for 8 weeks as compared to naphthaleneacetic acid and the control, according to table 2. Figure 2 gives a graphic presentation of these data.

The standard errors and least differences for significance between means are shown in the following tabulation:

Items	*5 percent*	*1 percent*
Hormone treatments		
Difference between highest and lowest	0.5	0.8
Difference between 2 adjacents	.4	.6
Standard error 0.12 with 6 d.f.		
Time periods		
Highest and lowest	.5	.7
Difference between 2 adjacents	.4	.6
Standard error 0.14 with 18 d.f.		
Between time intervals for same hormone treatment		
Highest and lowest	.9	1.1
Difference between 2 adjacents	.7	1.0
Standard error 0.24 with 18 d.f.		
Between any 2 hormones for same time interval		
Highest and lowest	.9	1.2
Difference between 2 adjacents	.7	1.0
Standard error 0.23		

These results suggest that treating the cuttings with indolebutyric acid will result in: 1, A higher percentage of rooted acerola cuttings; and 2, a better production of roots per cutting, which at the same time increases the probabilities of survival of these cuttings when transplanted to the cans or the field.

SUMMARY

Because of the high ascorbic acid content of the pulp of its fruit, the acerola (*Malpighia punicifolia L.*) has good potentialities as an economic crop for Puerto Rico. The Agricultural Experiment Station therefore, has selected clones of a high ascorbic acid content of which B. 15 is one of the best. The best and most rapid type of true-to-type propagation for the selected clones is by means of cuttings.

In this experiment cuttings of acerola clone B. 15 were treated with

indolebutyric and naphthaleneacetic acid and left in the sand beds for 6, 8, and 10 weeks. The major results were:

1. Indolebutyric acid significantly increased the number of cuttings that rooted.

2. Leaving the cuttings in the sand beds for 8 weeks proved this to be the optimum time interval for the rooting of the cuttings.

3. The use of the plant hormones significantly increased the number of roots per cutting.

RESUMEN

Por su alto contenido de ácido ascórbico, la acerola (*Malpighia punicifolia* L.) es una cosecha de gran potencialidad económica para Puerto Rico.

La Estación Experimental Agrícola de la Universidad de Puerto Rico ha venido seleccionando los tipos de este arbusto que producen frutas con el mayor contenido de ácido ascórbico. El método más rápido y eficiente para propagar estos tipos ha sido por medio de esquejes.

En el experimento que se ha informado aquí, se trataron los esquejes de acerola de la selección B. 15 con los ácidos indolbutírico y naftalinacético en polvo y luego se colocaron en propagadores de arena por períodos de 6, 8, y 10 semanas.

Los resultados fueron los siguientes:

1. El tratamiento de los esquejes con ácido indolbutírico aumentó significativamente el número de esquejes que arraigaron.

2. El dejar los esquejes en los propagadores de arena por un período de 8 semanas resultó ser el tiempo óptimo para que éstos arraigaran.

3. El uso de hormonas aumentó significativamente el número de raíces de los esquejes tratados.

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The Influence of the Age of Sugarcane on its Leaf-Nutrient (N-P-K) Content

George Samuels[1]

INTRODUCTION

The major plant nutrients (N-P-K) do not remain at a constant level throughout the life of the sugarcane plant. The level of a particular nutrient element in the cane leaf is influenced by the amount of that nutrient available to the plant from the soil and the presence and interaction with other nutrient elements in the soil and plant. But, aside from the supply of the nutrient itself, there are other important factors which influence the amount of nutrient found present in the cane plant. The more important of these factors are: Moisture, temperature, and age of the cane.

The influence of the age of the leaf or sheath sample on its nutrient content becomes an important consideration to the agronomist engaged in tissue analysis of sugarcane, whether using "foliar-diagnosis" or "crop-logging" systems. In foliar-diagnosis technique as practiced in Puerto Rico (*9*),[2] the object of the agronomist is to obtain sugarcane leaf samples at a cane age of 3 months. The reason is that the best correlation between nitrogen content of the leaf and cane yields per acre are obtained at this age. However, in commercial foliar-diagnosis practice, it has been found that leaf samples cannot always be taken at a cane age of 3 months. Then what influence does a delay of 1 or 2 months in sampling have on the foliar values obtained, as compared with a 3-month sampling? Are corrections necessary, and, if so, what are they?

Work done by Ayres (*1*) in Hawaii in the early 1930's indicated that there was a general decrease in the nutrient content of the sugarcane leaf with age. Clements in a detailed study in Hawaii followed the distribution of nitrogen in the cane plant with increasing age. He proposed in his crop-log procedure a Standard Nitrogen Index (SNI) in which the nitrogen level of the leaf could be standardized by use of a formula which took into consideration the moisture content of the leaf sheath and the age of the sample (*2*). Humbert and Howe (*5*), in an examination of 6 years' crop-logging information at Ewa Plantation, also found that nitrogen decreased with age

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[2] Italic numbers in parentheses refer to Literature Cited, pp. 169–70.

and sheath moisture. They presented a formula by which leaf nitrogen could be approximated at any combination of age and sheath moisture.

OBJECTIVE

However, aside from limited work by Samuels, Capó, and Bangdiwala (*8*) on the influence of moisture and age on leaf nitrogen, no information had been obtained in Puerto Rico as to the specific influence of the age of the cane on leaf nutrients (N-P-K) in sugarcane.

It was with the purpose of acquiring this information that the work reported here was carried out. This paper reports the results obtained on the influence of age on the leaf-nutrient values in sugarcane.

PROCEDURE

Sugarcane production in Puerto Rico, as in most cane-growing areas of the world, can be roughly classified as under or without irrigation. The majority of the unirrigated cane in Puerto Rico is grown in areas of abundant rainfall, so that water is not a main limiting factor in production. This cane receiving abundant rainfall will be called "nonirrigated cane" to distinguish it from "irrigated cane" growing in semiarid areas where rainfall is often a major limiting factor of growth.

Data were selected from sugarcane experiments in the nonirrigated and irrigated cane areas to determine the influence of the age of the cane on leaf-nutrient level. For the nonirrigated cane area, the data were derived mainly from three foliar-diagnosis experiments with sugarcane, plus a time-of-planting and harvesting experiment. The experiments were conducted at the Main Station Farm of the Agricultural Experiment Station at Río Piedras, P.R., the last under the leadership of P. Landrau, Jr., and covered the period 1954–57. The soil type used was a Vega Baja silty clay. The average yearly rainfall for this area is 73 inches. Sugarcane varieties B. 41227 and M. 336 were used in the foliar-diagnosis experiment; for the time-of-planting experiment P.R. 980 was employed. In the foliar-diagnosis experiment leaf samples were taken from three plant canes and two ratoons. Samplings were made approximately every 15 days from cane ages of 1 to 6 months. Twelve replications were taken each time for leaf samples. For the time-of-planting and harvesting experiment, leaf samples were taken from four replicates once every month beginning at 2 months and ending at 6 months of age.

The data for the irrigated cane were selected from a crop-logging experiment at Colonia Esperanza, Mercedita, and various crop-logs from commercial crop-logged cane at Luce and Co., Aguirre. The variety used for both sites was B. 37161, and the data covered the period from 1954–57. Leaf samples of the sugarcane were taken monthly beginning with a cane

age of 3 months and ending at 18 months for plant canes, and 12 months for ratoons. Eighteen replications were utilized for the crop-log experiment, while averages were obtained from 29 to 91 individual field crop-logs for the commercial crop-logging procedure.

The sugarcane leaf samples were prepared for anlyses as described in a previous publication (*9*).

RESULTS

The age of the sugarcane at the time of leaf sampling definitely had an influence on the nutrient level of nitrogen, phosphorus, and potassium in the leaf. The results of all experiments indicated that there was a general decreasing trend of leaf nutrients with increasing age of the cane. The decrease in leaf nutrients with age was very definite in the early stages of cane growth, say up to 6 months of age. After this the decrease continued for some fertilizer elements, whereas there were tendencies for nutrients to increase at a later cane age for others. However, even after these rises in nutrient levels, the leaf values at later ages never reached those found at the early ages of 2 to 3 months.

The findings of the influence of age of cane on the nutrient level in the leaf will be presented for each individual nutrient in order to give a more detailed picture of the results.

NITROGEN

The results of all experiments indicated that the nitrogen content of the leaf decreases as the age of the sugarcane increases (fig. 1). This decrease with age is not linear throughout the entire growing period because certain factors appear to have an influence on the nitrogen-age relationship. The use of irrigation, the type of crop—plant cane or ratoon, variety, and season of planting all have been found to modify the rate of decrease of leaf nitrogen with cane age.

Nonirrigated vs. Irrigated Sugarcane

The influence of the age of the cane on leaf nitrogen was found to be of different intensity for cane in nonirrigated areas as compared with irrigated areas. The rate of decrease was much steeper for nonirrigated than for irrigated cane (fig. 1). That is to say nitrogen values in the leaf dropped more rapidly per month of age for nonirrigated than for irrigated cane. A delay in sampling of 1 month produced an average decrease in the leaf nitrogen of 0.23 percent for humid cane, and of 0.09 percent for irrigated cane. The rate of decrease was about 2½ times as great for nonirrigated as for irrigated cane during the first 7 months.

Age-leaf nitrogen curves from Hawaiian data at Kohala (*6*) showed a

similar relationship of nonirrigated to irrigated cane. The rates of decrease of leaf nitrogen per month found from the Hawaiian curves were 0.085 percent for nonirrigated and 0.038 percent for irrigated cane. The rate of decrease was about 2¼ times as great for nonirrigated as for irrigated cane.

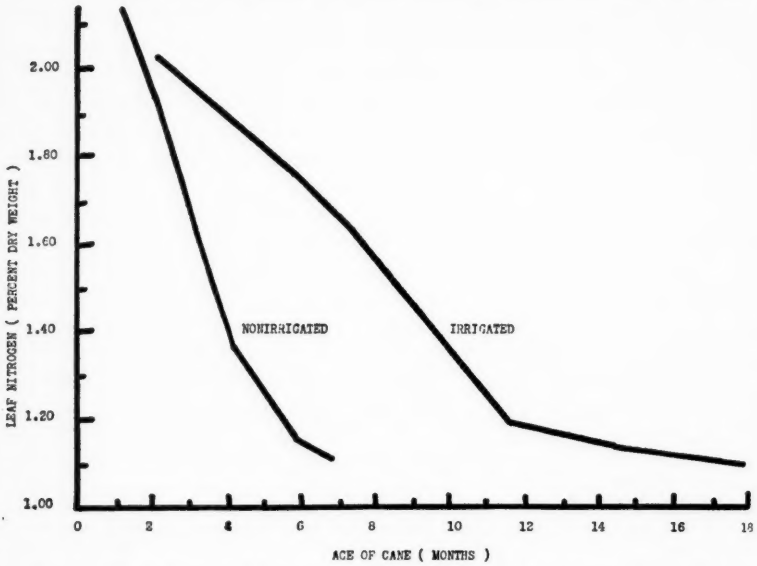

FIG. 1.—The influence of the age of irrigated and nonirrigated cane at sampling on its leaf-nitrogen content.

Type of Crop

The plant canes did not show the same rate of decrease for leaf nitrogen with age as did the ratoons. The leaf nitrogen declined much more rapidly in ratoons than in plant crops. This was true for both nonirrigated and irrigated sugarcane. For the first 6 months of growth the relation between leaf nitrogen and cane age showed a significant linear correlation which could be expressed in the following equations:

Nonirrigated cane:	Plant cane	$Yn = 2.31–0.152X$
	Ratoon	$Yn = 2.64–0.284X$
Irrigated cane:	Plant cane	$Yn = 1.94–0.076X$
	Ratoon	$Yn = 1.85–0.112X$

where Yn = percentage leaf-nitrogen content on a dry-weight basis and X = age of the sugarcane in months.

The loss of leaf nitrogen in nonirrigated ratoons was 0.28 percent as

compared with 0.15 percent for plant canes for each 1-month increase in age of the cane. With irrigated cane the loss per month was 0.11 percent of leaf nitrogen for ratoons as compared with 0.08 for plant cane. In both areas ratoons declined at an average rate 1¾ times faster than plant canes.

Varieties

Data for the varietal influence on the leaf-nitrogen-age relationship were available only for nonirrigated cane. There was no significant difference in the rate of decrease in leaf nitrogen for the three varieties tested: B. 41227, M. 336, and P.R. 980.

Although no varietal age-nitrogen data were available for irrigated canes

TABLE 1.—*The decrease in leaf-nitrogen per month for sugarcane-leaf samples taken at various dates throughout the year*

Planting date	Period covered by leaf sampling	Times leaf samples were taken	Decrease in leaf-nitrogen values per month on a dry-weight basis
		Number	*Percent*
May 28	Aug. 18 to Dec. 8	5	0.19
June 27	Aug. 31 to Dec. 27	5	.17
July 26	Oct. 1 to Jan. 23	5	.20
Aug. 23	Oct. 24 to Feb. 20	5	.20
Sept. 20	Nov. 26 to Mar. 28	5	.23
Oct. 18	Jan. 23 to Apr. 23	4	.23
Nov. 15	Feb. 20 to May 21	4	.22
Dec. 13	Feb. 28 to June 8	4	.22
Feb. 7	May 21 to July 13	3	.17

in Puerto Rico, an examination of the crop-log data in a study at the Hawaiian Commercial and Sugar Co., Ltd., Hawaii (7), showed that there were no significant differences in the slopes of nitrogen-age linear equations for Hawaiian varieties 328560, 371933, and 382915 for the first 12 months of age.

Time of Year

It did not seem that the time of the year when the leaf samples were taken had any appreciable influence on the nitrogen-age relationship for nonirrigated sugarcane in Puerto Rico. Utilizing a time-of-planting and harvesting sugarcane experiment, leaf samples taken at various grouped intervals throughout the year revealed no significant difference in the slopes of their linear regressions. The changes in leaf nitrogen per month found for these various sampling intervals are given in table 1. Although the slopes of the linear regression were not significantly different when all

sampling periods were compared, there was a tendency for the lower rates of decrease of nitrogen per month to be associated with samples taken during the period June to December.

The time of the year sugarcane leaf samples are taken was found to influence leaf-nitrogen values, lower values being associated with higher temperatures. Credit for such influence has previously been given to temperature (*4*). In the spring and summer leaf-nitrogen values are generally lower and the rate of drop is not so great as in the winter when leaf-nitrogen

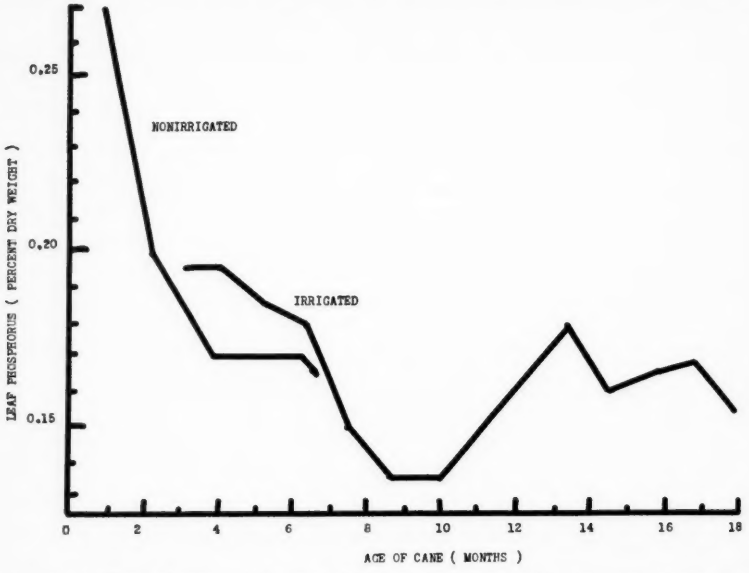

FIG. 2.—The influence of the age of irrigated and nonirrigated cane at sampling on its leaf-phosphorus content.

values are higher. It appears, however, that these influences were not of sufficient magnitude in Puerto Rico significantly to influence the leaf-nitrogen-age relationship.

PHOSPHORUS

The general relationship between leaf phosphorus and age of the sugarcane is shown in figure 2.

Nonirrigated sugarcane showed a sharp decrease in leaf phosphorus from the ages of 1 to 3 months. The drop in leaf phosphorus from 1 to 2 months was about 0.07 percent. At first inspection this does not seem too high a value; but, considering that normal leaf-phosphorus values are about 0.18

percent at 3 months, it represents a large decrease. The rate of decline was less steep from the ages of about 2 to 6 months, and had decreased to about 0.015-percent leaf phosphorus per month. There was no apparent decrease in leaf phosphorus from the ages of 4 to 6 months. Another decrease in leaf phosphorus occurred between 6 to 7 months, when the study was concluded; it amounted to 0.01 percent for that month.

The rate of decrease for leaf phosphorus was not so rapid in the early months for irrigated as for nonirrigated cane. From 3 to 4 months of age there was no change, and from 4 to 6 months the decrease amounted to about 0.008 percent of leaf phosphorus per month. A more rapid decline was encountered in the 6- to 9-month period, amounting to a decrease of about 0.02-percent leaf phosphorus per month. There was an increase in leaf phosphorus from 10 to 13 months, so that levels in the leaf equalled those found at 6 months. The leaf phosphorus dropped irregularly after 13 months. Data from Hawaii on irrigated cane also indicated that sheath-phosphorus values decreased until a cane age of about 10 months, then increased to about 13 months.

No differences in rate of change of leaf-phosphorus values per month for type of crop were apparent as for leaf nitrogen. The data for plant canes tended to be highly variable, and none of the linear regressions obtained was significant. The relationship between leaf phosphorus and age for the first 6 months was more marked in ratoons and the linear regressions obtained generally proved significant.

Variety differences were not evident in the rate of decrease of leaf phosphorus with age for the nonirrigated canes studied, *i.e.* B. 41227, M. 336, and P.R. 980.

POTASSIUM

Leaf potassium did not decrease with age throughout the whole growing period of the sugarcane whether irrigated or not. At first there was a decrease in leaf potassium with increasing age, as was found for leaf nitrogen and phosphorus. However, there was a definite increase in leaf potassium as the cane neared maturity. The variations of leaf potassium with age is shown in figure 3.

There was a sharp decrease in leaf potassium in nonirrigated cane up to an age of 4 months which amounted to about 0.24 percent per month. Between ages of 4 to 5 months the decrease was very much less, being only 0.02 percent of leaf potassium. After 5 months of age the leaf potassium increased; it rose at a rate of around 0.08 percent per month, until about 8 months, at which time the study was concluded.

The pattern of the age-leaf potassium curve for irrigated cane was very similar to that for the nonirrigated cane for the first 6 months. The rate of

decline of leaf potassium was not as sharp for the irrigated as for the nonirrigated cane. It was about 0.18 percent of leaf potassium per month for a cane age of 3 to 5 months. Aside from brief rises at 6 and again at 10 months, the leaf potassium declined rapidly until a cane age of 14½ months was attained. Then the leaf potassium rose until a cane age of 18 months. In the period from 3 to 14 months the leaf potassium dropped a

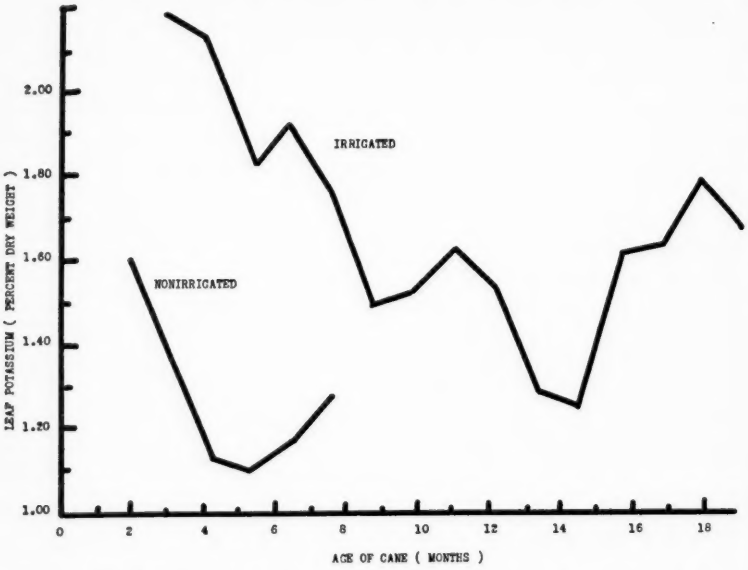

FIG. 3.—The influence of the age of irrigated and nonirrigated cane at sampling on its leaf-potassium content.

total of 0.93 percent in about 11 months. Similar curves were obtained for sheath potassium and age in Hawaii.

DISCUSSION

The data presented show that the age of the cane does influence the leaf-nutrient content in Puerto Rico. Leaf-nitrogen levels drop as the age of the cane increases. This relationship is significant, its rate appearing to be modified by type of crop, plant cane or ratoon, and water availability, *i.e.* nonirrigated or irrigated cane. Phosphorus values do not continue to decrease regularly in the leaf throughout the growth of sugarcane. However, decreases in leaf phosphorus are of sufficient importance in the early months to merit attention. The behavior of leaf potassium with age appears to be somewhat similar to that of leaf phosphorus.

Sufficient evidence has been collected to be used in making corrections for the influence of age on the leaf-nutrient content. In Puerto Rico, where foliar-diagnosis leaf samples are standardized at a cane age of 3 months, samples taken later than this should be corrected. Inasmuch as most sampling here is limited to a period of about 6 months because of the

TABLE 2.—*Correction factors (percent) for the influence of age of the leaf sample on nutrient content*[1]

Nutrient	Type of crop	Correction factor for age of leaf sample[2]					
		Nonirrigated cane			Irrigated cane		
		4 months	5 months	6 months	4 months	5 months	6 months
Nitrogen	Plant cane	0.15	0.30	0.45	0.08	0.15	0.23
	Ratoon	.28	.56	.74	.11	.22	.33
Phosphorus	Plant cane or ratoon	.015	.015	.015	0	.008	.016
Potassium	do.	.24	.24	.24	.12	.24	.36

[1] These values are based on the variation of leaf nutrient for cane up to a cane age of 6 months.

[2] As the standard age of the cane for leaf sampling is 3 months of age, the correction factors are to be added to the leaf-nutrient value obtained when sampled beyond 3 months and up to 6 months of age.

TABLE 3.—*Average standard leaf-nutrient values (percent) for sugarcane in Puerto Rico in general on a fertility-status basis*

Fertility status	Leaf-nutrient content, dry-weight basis, of		
	N	P	K
Very low	<1.00	<0.10	<1.00
Low	1.00–1.40	0.10–.15	1.00–1.40
Slightly low	1.40–1.55	.15–.18	1.40–1.55
Normal	1.55–2.00	.18–.24	1.55–2.00
High	2.00–2.50	.24–3.0	2.00–3.00
Very high	>2.50	>0.30	>3.00

closing-in of the cane and its shorter growing period—average 12 months—these correction factors can best be applied for samples taken up to 6 months. As the leaf-nutrient values decrease with age the correction factors should be added to the leaf values obtained. To assist workers engaged in foliar diagnosis of sugarcane, the suggested correction factors for age of sampling are presented in table 2 and the standard values for 3 months are given in table 3. For ages beyond 6 months approximate rates of nutrient loss per month can be calculated from the curves presented in figures 1, 2, and 3.

It is not to be assumed that the suggested values presented are final. Further work is still needed, especially on phosphorus and potassium.

It would be misleading to give the impression that the age of the sugarcane exerts the greatest influence on nutrient changes in the leaf, aside from the supply of nutrient itself. Moisture cannot be omitted from the picture as to either its direct or indirect effects. In an excellent study of factors affecting the growth of sugarcane Clements (*3*) showed that leaf nitrogen had a highly significant correlation with sheath moisture and age of cane, the nitrogen correlation with moisture being +0.797 and with age −0.684, and the sheath-moisture correlation with age being +0.646. As the cane plant grows older its sheath-moisture and leaf-nutrient (especially nitrogen) values drop. Clements found that the two dominant factors affecting the level of sheath phosphorus were sheath moisture and total sugars. It appears that age was not of significant importance in its effects on sheath-phosphorus levels in irrigated cane in Hawaii.

SUMMARY

The age of sugarcane exerts a definite influence on the nutrient content of the sugarcane leaf. The effects of age on the leaf-nutrient (N-P-K) content were as follows:

1. There was a decrease in the nitrogen content of the leaf as the cane age increased.

2. The rate of decrease of nitrogen with age was greater for nonirrigated than for irrigated cane, being about 2½ times as large for the former as for the latter during the first 7 months.

3. Leaf nitrogen declined more rapidly in ratoons than in plant crops.

4. There was no significant difference in rate of nitrogen decline for the varieties tested: B. 41227, M. 336, and P. R. 980.

5. The time of year the leaf samples were taken had no significant effect on the rate of leaf-nitrogen decline with the age of cane.

6. There was a marked decrease in leaf phosphorus with age of cane in nonirrigated cane up to 4 months, and no change from 4 to 6 months.

7. Irrigated cane decreased in leaf phosphorus, but not quite so rapidly as nonirrigated cane, a decrease apparent until about 9 months. After this the leaf phosphorus began to increase until a cane age of about 14 months, where it once again began decreasing.

8. Leaf potassium did not decrease with age throughout the whole growing period of the sugarcane. At first there was a decrease in leaf potassium with increasing age; however, there was a definite increase as the cane neared maturity. This increase began at about 5½ months for nonirrigated and 15 months for irrigated cane.

9. A table of suggested correction factors for age of sampling is presented to assist workers engaged in foliar diagnosis of sugarcane.

RESUMEN

La edad de la planta de caña de azúcar ejerce una influencia definitiva sobre el contenido de nutrientes en sus hojas. Los efectos de la edad sobre el contenido de los nutrientes (N-P-K) se explican a continuación:

1. Hubo una merma del contenido de nitrógeno en la hoja, a medida que aumentaba la edad de la caña.

2. La proporción en que mermó el contenido de nitrógeno según la edad de la planta fué mayor en la caña sin riego que en la que se regó. Durante los primeros siete meses, la proporción de esta merma fué 2½ veces mayor en la caña sin riego.

3. El nitrógeno en la hoja se redujo más rápidamente en los retoños que en las cañas de plantilla.

4. No hubo una diferencia significativa en cuanto a la proporción de la merma en el contenido de nitrógeno en la hoja para las variedades bajo prueba: B. 41227, M. 336 y P.R. 980.

5. La época del año en que se tomaron las muestras de hoja no demostró ejercer un efecto significativo sobre la proporción de la merma en el contenido de nitrógeno en la hoja con la edad de la caña.

6. Hubo una merma notable en el contenido de fósforo en la hoja relacionada con la edad de la caña sin riego hasta los 4 meses; no se registró cambio alguno de los 4 a los 6 meses.

7. La caña bajo riego sufrió merma en el contenido de fósforo de la hoja, pero no tan rápidamente como lo fué en la caña que no se regó. Esta merma no fué aparente hasta los 9 meses. Después de tener dicha edad, el contenido de fósforo comenzó a aumentar hasta que la caña llegó a los 14 meses, luego de lo cual empezó de nuevo a reducirse.

8. El contenido total de potasio en la hoja no mermó con la edad de la caña a través de todo el período de crecimiento de la planta. Al principio hubo una reducción según avanzaba el tiempo; pero ésta fué compensada definitivamente por un aumento continuado a medida que la planta alcanzaba su madurez. Este aumento comenzó a los 5½ meses en la caña sin riego y a los 15 meses en la caña bajo riego.

9. Se presenta un cuadro con factores de corrección para edad que pueden aplicarse cuando se tomen las muestras y que servirá de ayuda a quienes se dediquen a hacer el diagnóstico foliar de la caña de azúcar.

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Evidence of the Presence of the Tobacco-Etch Virus in Puerto Rico[1]

L. López Matos[2]

INTRODUCTION

Cigar-filler and chewing-tobacco plants presenting chlorotic and necrotic spots have been found in many fields in the cigar-filler and chewing-tobacco areas of Puerto Rico during the past 6 years. The number of affected plants increases by the end of the tobacco season, especially among tobacco planted in December or later. Very few cases were observed in the field at the beginning of the tobacco season. In only two cases have plants showing these symptoms been found in seedbeds since 1954.

Chewing tobacco is more severely affected. This can be accounted for by the fact that chewing tobacco is topped earlier and the leaves are left for a longer period until they are mature and the plant is ready for harvest. Plants infected at an early stage of growth show many necrotic spots, arcs, or rings. Soon after the tobacco is topped the necrotic areas coalesce, form, ing holes that again coalesce, practically destroying the leaf (fig. 1, A, B, and C). Chewing-tobacco leaves affected in this way are useless. The tobacco producer includes this disease in the group of diseases that he refers to as *tabaco pinto*, which is a general term applied to tobacco leaves with any type of lesion on them. The author has been referring to this disease as the *salpicado del tabaco* to distinguish it from other virus diseases.

Thinking it possible that this disease might be caused by the etch virus, studies were initiated to identify the virus. This paper reports work on the identification of the virus.

SYMPTOMS

The variation in the cigar-filler and chewing-tobacco varieties in Puerto Rico presents difficulties in the description of the symptoms of tobacco-etch virus. Plants have been found showing symptoms very similar to those described by Johnson (*3*)[3] for different strains of the etch virus, such as "etch", "etch + ", "severe etch", and "coarse etch" found in Kentucky and Georgia. Some field-infected plants present fine etching on older leaves (fig. 1,D). Some of these plants also present suckers with chlorotic spots

[1] Contribution from the Gurabo Substation.

[2] Assistant Pathologist, Agricultural Experiment Station, University of Puerto Rico, Gurabo, P.R. Thanks are due J. Enrique Pérez for carrying out the serological tests.

[3] Italic numbers in parentheses refer to Literature Cited, p. 181.

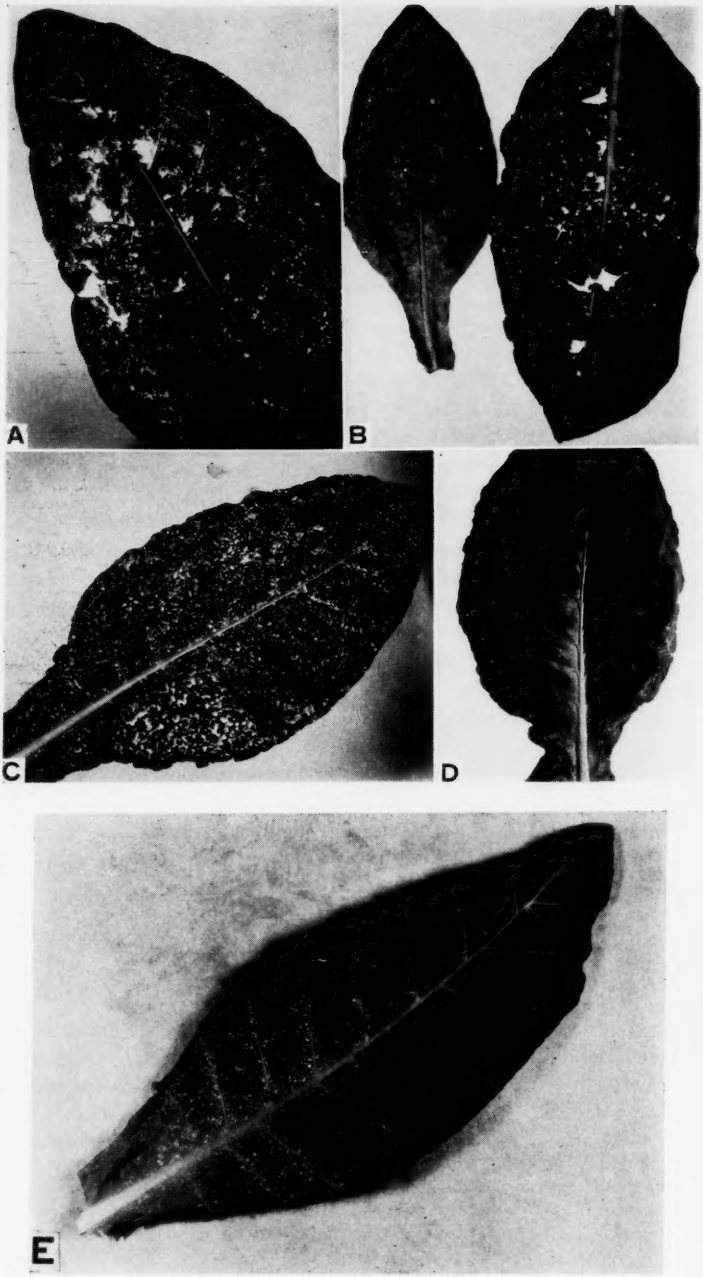

FIG. 1.—Tobacco leaves showing symptoms of the virus disease: A, B, and C are leaves from field-infected plants showing necrotic areas and holes resulting from coalescence of small necrotic spots and arcs and/or rings. D and E show leaves from field-infected plants showing fine etch and arcs.

and arcs or lines in the younger leaves. Some of these spots become reddish-brown and then gray or white as the leaf becomes older. Other plants present larger necrotic spots and arcs, or small rings in the older leaves (fig. 1,E). These symptoms may be found alone or accompanied by vein necrosis in some plants (fig. 1,A). Other plants may show vein necrosis with a few necrotic spots (fig. 2,A). Others still show more necrotic rings than necrotic spots in the older leaves. Some of these plants may also present chlorotic spots and arcs in the younger leaves.

Plants may have all leaves affected or only one or two. Other plants may show two older leaves with etch symptoms and three or four leaves free of spots, and then two or three of the younger leaves with chlorotic, rusty-brown, or necrotic spots. Some plants may show severe symptoms of etch plus distortion of the younger leaves and stunting. However, these cases are very rare.

Figure 2,B shows a leaf of a field-infected plant presenting many small necrotic rings. The sap of this plant was rubbed on the leaves of small seedlings of tobacco variety Virginia 12. Figure 2,C shows one of the seedlings with vein necrosis and necrotic arcs and spots in the inoculated leaves and chlorotic and necrotic or reddish-brown spots and arcs in the younger leaves and a tendency of these leaves to curve back.

Figure 2,D shows the same plant 3 weeks later. This plant presented chlorotic spots and arcs in the younger leaves. As the leaf became older these spots became rusty brown and then gray or whitish. The borders of the leaves also show the tendency to curve back. This tendency is more marked in inoculated plants. Not all plants inoculated with the same inoculum present symptoms in the same fashion. Symptoms are more marked in some plants than in others. Very few chlorotic spots are found on some plants. More chlorotic spots may appear on these plants. If the plants are kept growing vigorously at all times, chlorotic and rusty-brown or necrotic spots can be found at any time.

MATERIALS AND METHODS

Plants used in these studies were grown in clay pots in the greenhouse using steam-sterilized soil with the same amount of fertilizer per plant. Insects were controlled with sprays of Parathion (15 percent) at the rate of 1 pound per 50 gallons of water, applied once every other day. Silicon carbide grit No. 400 was used as an abrasive when virus transmissions were made by rubbing the sap of infected plants on the leaves of healthy ones. Plants were inoculated when they had three to four leaves and were growing vigorously.

The study of the virus was made by means of differential hosts, following the key proposed by Valleau (*6*), and, serologically, by following the precipitin reaction test described by Bawden (*1*).

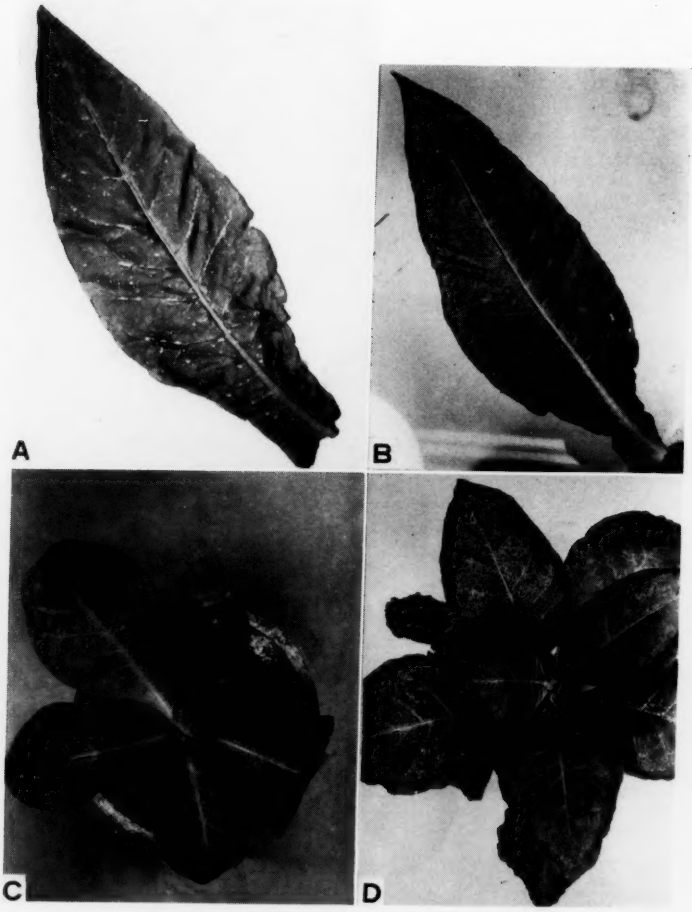

FIG. 2.—Tobacco leaves showing symptoms of the virus disease: A, Leaf from a field-infected plant showing vein necrosis and a few necrotic spots. B, Leaf from a field-infected plant showing many necrotic ares and small rings. C, A tobacco seedling inoculated with sap obtained from the leaf of the field-infected plant shown in B. The inoculated leaf presents fine vein necrosis, the younger leaves present chlorotic spots and ares, and stunting. D, The same plant as in C 3 weeks later.

The following operations were made following the key proposed by Valleau:

1. Determination of the destruction of the infectivity of the virus by air-drying the infected leaves: The infected tobacco leaves were air-dried at room temperature. Then they were crushed in water using approximately twice as much water as the volume of the crushed leaves. The extract was rubbed on the leaves of healthy plants of cigar-filler tobacco variety Virginia 12. Fifteen plants were inoculated. An equal number of plants were left uninoculated and used as a control. Fifteen plants of tobacco hybrids carrying the *Glutinosa* type of resistance to the common tobacco-mosaic virus were also inoculated to insure that the tobacco-mosaic virus was not present.

2. Determination of transmission of the virus by plant sap: Juice obtained from samples of field-infected plants of both chewing- and cigar-filler tobacco brought from different parts of the tobacco-growing area was rubbed on the leaves of healthy plants of cigar-filler tobacco. Table 1 indicates the place of origin of the sample and the variety of tobacco from which it was obtained.

3. Infectivity on cucumbers: Leaves of cucumber of the variety Puerto Rico 39 were rubbed with sap from each of the leaf samples obtained in the field.

Twelve leaf samples from plants having symptoms of etch were tested serologically. Table 1 gives the description of each sample tested and the results of the tests. Five samples were taken from plants inoculated in the greenhouse. The inoculum for these plants had been obtained from the same plant from which sample No. 1 in table 1 was obtained. The other seven samples (Nos. 2 to 8 in table 1) were taken from suckers of field-infected plants. The samples were ground in clean sterilized mortars and the crude sap extracted by squeezing each macerate through pieces of double-layer cheesecloth. The saps were then clarified by low-speed centrifugation. Each sap was mixed separately in a precipitin tube with specific antisera for the virus of tobacco severe etch, tobacco mosaic, pepper mosaic, tobacco ringspot, potato virus X, and normal rabbit serum.

Insect-transmission tests were also made using the aphid *Myzus persicae* Sulz. as the vector. Table 2 shows the results obtained in the six greenhouse trials made.

No attempt was made to control temperature and humidity. The seedbeds were kept in insect cages. In trials Nos. 1 to 5 the plants were grown in 4-inch clay pots. Fifteen plants were used in trials Nos. 1 to 4. Forty plants were used in trial No. 5. All plants in trials Nos. 1, 2, and 5 were kept in insect cages 4′6″ wide; 2′6″ long; and 3′ high, consisting of Lumite in an aluminum frame (fig. 3,A). In trials Nos. 1 and 2, plants were about

TABLE 1.—*Results of the inoculation of the virus in tobacco varieties and in cucumber, and place of origin of the sample and its serological reaction*

Sample No.	Variety and type of tobacco	Place of origin	Number of plants of Virginia 12 inoculated and infected	Symptoms in Virginia 12	Plants of cucumber inoculated[1]	Serological reaction[2]			
						Samples Tested	TMV[3]	MV[4]	Etch
1	*Tabaco del país*, chewing	Barrio Coto, Isabela, Francisco Chávez's Farm	5	Etching	6	5	−	−	3+ 2−
2	Virginia 12, cigar filler	Barrio Cañabón, Caguas, Station Farm	6	do.	9	1	−	−	+
3	do.	Barrio Rincón, Gurabo, Station Farm	4	do.	6	1	−	−	−
4	*Orama*, cigar filler	Barrio Cordilleras, Ciales	6	do.	6	1	−	−	+
5	*Tabaco del país*, chewing	Barrio Terranova-Quebradillas	6	do.	6	1	−	−	−
6	*Tabaco del país*, chewing	Barrio Coto, Isabela	4	do.	6	1	−	−	+
7	do.	Barrio Guerrero, Isabela, M.A. Guevara's Farm	4	do.	6	1	−	−	−
8	Chewing tobacco hybrid	Station Farm, Isabela	4	do.	6	1	−	−	−

[1] No plant became infected. No cucumbers displayed symptoms.

[2] Serological test was made by using a sample taken from the top leaves of field-infected plants, except in sample No. 1 in which the 5 samples were taken from plants which had been inoculated in the greenhouse, the original plant having been found at the farm of Don Francisco Chávez at Barrio Coto, Isabela.

[3] TMV = tobacco mosaic virus.

[4] MV = mottle virus.

6 inches in height and growing vigorously. In trials Nos. 3 and 4 the plants were grown in insect cages 24″ wide; 17½″ long; and 10½″ high, consisting of Lumite with a glass window in a wooden frame (fig. 3,B). In trials Nos. 3 and 4, insects were placed when the plants were about 3 inches in height. In trial No. 5 the insects were placed when the plants were about 6 inches in height. In trial No. 6 the plants were grown in 2-inch clay pots, and each plant was protected with individual insect cages. The cages were

TABLE 2.—*Results in tests for the transmission of the etch virus to Virginia 12 variety by placing 25 aphids* (Myzus persicae *Sulz.*) *on each plant*

Trial No.	Numb of plants	Date of inoculation	Number of infected plants	Date of first symptom[1]	Symptoms	Remarks
1	15	Nov. 17, '54	1	6 days	Etching	Plants near 6″ in height
2	15	Dec. 9, '54	2	1 at 6 days 1 at 7 days	do. do.	Do.
3	15	Jan. 8, '55	0	—	—	Plants near 3″ in height
4	15	Jan. 8, '55	2	1 at 6 days 1 at 5 days	do.	Do.
5	40	Jan. 23, '55	2	1 at 9 days 1 at 11 days		Plants were from same group as in 3 and 4 and not growing vigorously near 6″ in height
6	16	Jan. 15, '58	5	8 days	do.	Plants were very small. Leaves were the size of a 10 cent coin or smaller.

[1] Indicates the number of days after inoculation that each of the plants, the numbers of which appear in the preceding column, developed the first symptoms.

2″ wide, 2″ long, and 7″ high, consisting of plastic sides and the top of Lumite. The top had a small hole with a cork stopper (fig. 3,C).

Twenty-five insects per plant were used in all trials. The insects were kept for 72 hours feeding on the infected plants. After feeding they were fasted for half an hour and placed on the healthy plants for 24 hours. To transfer the insects from the infected to the healthy plants, they were collected with a small sterile camel-hair brush and placed in a sterile petri dish. After fasting for 30 minutes the aphids were placed on the leaves of the healthy plants by means of another small sterile camel-hair brush. The leaves of the plants to which the insects were transferred were approximately the size of a 10 cent coin, or less. After feeding for 24 hours on the healthy plants the insects were killed with a spray of 15-percent Parathion, at the rate of 1 pound per 50 gallons of water.

farm of Don Francisco Chávez at Barrio Coto, Isabela.
[3] TMV = tobacco mosaic virus.
[4] MV = mottle virus.

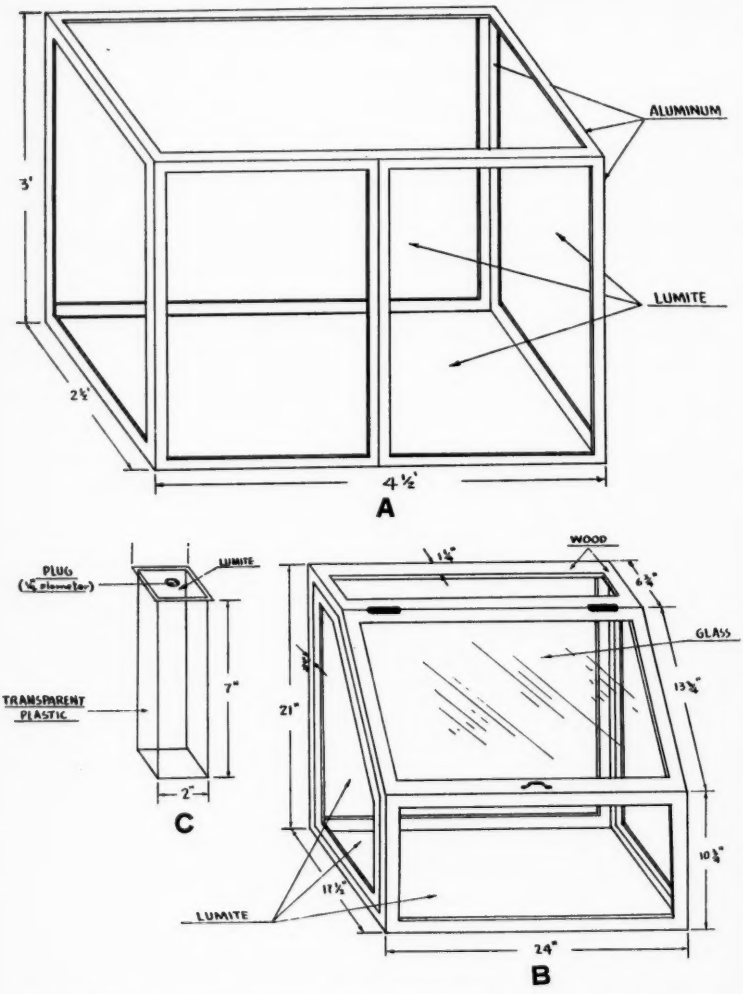

FIG. 3.—A, B, and C: Drawings of three types of insect cages used for insect transmission of the virus.

Serological tests and differential host plants such as Holmes' Samsoun tobacco and Large Bell Hot pepper were used to confirm the presence of the virus under study and to eliminate the possibility of having another virus present, such as the tobacco-mosaic virus or the tobacco-mottle virus.

The Holmes' Samsoun tobacco carries the factor for resistance to the tobacco-mosaic virus present in *Nicotiana glutinosa* (Holmes 1938) (*2*). The leaves develop necrotic lesions when inoculated with the tobacco-mosaic virus. *Capsicum frutescens* var. Large Bell Hot develops systemic necrosis followed by defoliation and death of the plant when inoculated with the tobacco-mottle virus (Roque and Adsuar 1941) (*5*). The differential host plants were used in trials Nos. 1 to 5. Both the differential host plants and the serological tests were used in trial No. 6. Serological tests were made to detect the presence of the following viruses: Tobacco ringspot, tobacco mosaic, potato X, and the pepper mosaic, besides severe etch.

RESULTS AND CONCLUSIONS

No symptoms were produced on plants of *Nicotiana tabacum* var. Virginia 12 nor of the Holmes' Samsoun variety inoculated with the juice extracted from air-dried leaves of infected plants. This indicated that the tobacco-mosaic virus was not present.

The virus is easily transmitted to tobacco by plant sap. *N. tabacum* var. Virginia 12 developed symptoms very similar to those observed in field-infected plants. The symptoms were more marked in some plants. *N. tabacum* var. Holmes' Samsoun presented vein-clearing and light chlorosis of the younger leaves. Tiny necrotic rings appeared as the leaves became older. These symptoms were followed by the presence of necrotic spots, arcs, and rings. Again, some of the plants presented more marked symptoms than others. Similar symptoms were observed with all samples of field-infected plants. The absence of infectivity of necrotic lesions typical of tobacco-mosaic virus on Holmes' Samsoun tobacco showed that the virus causing the disease was not the tobacco-mosaic virus. No symptoms were produced on cucumber, *Cucumis sativus* var. Puerto Rico 39, inoculated with the virus with any of the samples tested.

Plants inoculated with juice of plants from samples Nos. 2 and 3 did not produce any infection on peppers, *Capsicum frutescens* var. Large Bell Hot, while juice from plants of samples Nos. 1, 4, 5, 6, 7, and 8 produced mottling and distortion of the leaves. Absence of systemic necrosis followed by death of the plants indicated that the pepper-mosaic and tobacco-mottle virus were not present.

Three of the seven samples of field-infected plants tested serologically gave a strong precipitate with the serum against the etch virus. Of the five plants which had been inoculated with juice extracted from a field-infected plant, the samples of three also gave a strong precipitate with the serum against the etch virus. None of the samples reacted with any of the other viral antisera used in the tests, nor with normal rabbit serum.

The virus under study was found to be transmitted by the aphid, *Myzus*

persicae. In five out of six trials the disease was transmitted. Results are given in table 2. First symptoms developed from 5 to 11 days after placing the insects on the healthy plants. The percentage of infection varied from 4 to 31. Kassanis (*4*) found that severe etch virus is transmitted by *Myzus persicae* and *M. circunflexus*.

From the results of the test made following Valleau's method of identification of viruses in tobacco and from the results of the serological tests, it is concluded that the virus that causes *el salpicado del tabaco* in Puerto Rico by developing chlorotic spots followed by necrotic spots, arcs, and rings in the leaves, is related to the etch virus. It is also concluded that besides the severe-etch strain we may have the mild strain and possibly the coarse-etch strains.

SUMMARY

A new virus disease was found in cigar-filler and chewing-tobacco plants in Puerto Rico, in 1954. The infected plants developed chlorotic spots on the younger leaves followed by the presence of necrotic spots, arcs, and sometimes rings as the leaves became older. The younger leaves also appeared slightly chlorotic. Chewing tobacco is more severely affected than cigar-filler tobacco. The virus is easily transmitted mechanically by plant sap. It was also found to be transmitted by the aphid *Myzus persicae*. The virus was identified by using the method proposed by Valleau (*6*) to identify viruses that are found causing disease in commercial plantings of tobacco, and by serology, using the method recommended by Bawden (*1*). Antisera for tobacco severe-etch virus was used. It was found that the virus that causes what is referred to as *el salpicado del tabaco* in Puerto Rico is related to the etch virus. The variety of symptoms observed suggest that besides severe etch the mild- and coarse-etch strains of the virus may also be present in Puerto Rico.

RESUMEN

Una nueva enfermedad virosa que ataca las plantas de tabaco para tripa y para hilar se encontró en Puerto Rico en 1954. Las plantas atacadas por esta enfermedad desarrollan manchas cloróticas en sus hojas seguidas de puntos, arcos y, a veces, pequeños anillos necróticos. Las hojas más tiernas se tornan ligeramente cloróticas. Es el tabaco de mascar el que se afecta más severamente debido a esta enfermedad. El autor ha llamado esta enfermedad el salpicado del tabaco para así diferenciarla de otras enfermedades también causadas por otros virus.

Al principio de la cosecha se encontraron casos aislados de plantas atacadas por la enfermedad en los plantíos. Se notó que la incidencia aumenta en las siembras tardías o sea las que se hacen en diciembre, o más tarde. Tal parece que la enfermedad se ha extendido durante los últimos años.

El virus que causa esta enfermedad se trasmite mecánicamente por medio de la savia. También se encontró que el agente que la trasmite es el áfido *Myzus persicae* Sulz.

El virus se identificó por medio del método propuesto por Valleau (*6*) para identificar los virus que causan enfermedades en los plantíos comerciales de tabaco y también por medio de los métodos serológicos recomendados por Bawden (*1*). En la prueba se usó un antisuero para el virus que causa el "severe etch" al tabaco. Se encontró que el virus que causa el salpicado del tabaco en Puerto Rico es una cepa del virus "etch". La variación en los síntomas observados indican que hay la posibilidad de que existan varias cepas del virus "etch" en Puerto Rico.

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Phosphate Retention By Muck Soils[1]

Julián Roldán[2]

INTRODUCTION

The subject of phosphate retention, meaning the removal of phosphate from a solution by a soil or by a soil constituent, has been widely studied in soil science. Logically, much of the emphasis has been placed on mineral soils, for they make up the majority of the soils on earth. By comparison, therefore, the work done on phosphate retention by an organic soil seems to be very slight.

LITERATURE REVIEW

Doughty (*4*)[3], determining the retention capacity of peat soils for phosphorus at various pH levels, found that retention was at maximum at pH 2.0. A gradual decrease in retention occurred as the pH was increased. In determining the effect of leaching peat with acid on phosphate retention the data obtained showed that it decreased as compared with a water-leached peat, when fixation was measured at the same pH value in both cases.

To test the effect of iron absorption on phosphate retention he saturated the peat with iron; an increase in its phosphate-retention capacity was observed. Retention was a maximum at pH 2.5. A decrease was observed with a change of pH in either direction. The data were similar for an aluminum-saturated peat, but the maximum phosphate retention was at pH 4.5.

Evidence was obtained showing that water-soluble salts had considerable effects between pH's 6 and 8. Doughty finally reached the conclusion that the formation of iron, aluminum, and calcium phosphates would account for the retention of phosphate under field conditions.

Further studies carried on by Doughty (*5*) confirmed his previous findings, in that leaching with hydrochloric acid reduced the retention capacity of the peat, the extent being dependent on the normality of the acid used.

[1] Part of a thesis submitted to the Faculty of the Graduate School of Cornell University, Ithaca, N. Y., in partial fulfillment of the requirements for the degree of Master of Science.

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[3] Italic numbers in parentheses refer to Literature Cited, p. 193.

Weidemann (*12*) found that the very acid mucks he studied showed negative retention of phosphorus, but the less acid mucks showed positive retention. Dunton (*6*), in phosphate-retention studies, proved that the addition of humus was effective in decreasing the fixation of the applied phosphorus. Burd (*1*), working with displaced soil solutions, suggested that organic matter does not adsorb phosphate, or if it does, the phosphate is held by such weak linkages that it is readily released to the soil solution or plant.

Dalton, *et al.* (*2*) stated that the principal products of phosphate fixation in acid soils formed by the reactions of soluble phosphates with iron and aluminum are relatively insoluble. Wild (*13*), in his review of phosphate retention by soils, asserted that phosphate ions may be retained by an exchange reaction with the hydroxyl ions that are normally linked with the iron and aluminum ions. Miller (*10*), through his work on phosphate retention by hydrated alumina, came to the conclusion that the mechanism by means of which hydrated ferric oxide retains phosphate is similar to that for hydrated aluminum oxide. Moser (*11*) found that there is a maximum phosphate fixation between pH 4.5 and 6.0. Ghosh and Bhattacharyya (*7*), working with hydrous gels of alumina and silica, demonstrated that phosphate ions are removed from solutions by forces of electrical attraction. Davis (*3*) reported that the degree of retention was dependent upon the pH of the applied solution.

Haseman, Brown, and Whitt (*8*) ascribed phosphorus retention in soils to certain calcium phosphate-exchange combinations with clay and hydrous oxides of iron and aluminum, and to phosphates of iron and aluminum. However, they emphasized that the exact nature of the reactions of phosphorus with the soil constituents was poorly understood because of the difficulty with which the products of the fixation reactions are detected and identified in soil systems.

PHOSPHATE-RETENTION STUDIES

PREPARATION OF SAMPLES

A peat-soil sample derived from low-ash vegetation was obtained from Orange County, N. Y. This soil had an original pH of 3.8. After the peat was limed a 1:2 soil-water suspension gave a pH of 5.15. The exchange capacity of the peat soil was 196 m.e. per 100 gm. of soil. At a pH of 5.15 the base saturation was 26 percent.

An air-dried sample of the peat was ground in a hammer-mill and part of it was used for the phosphate-retention studies on the untreated peat.

PEAT TREATED WITH $Ca(OH)_2$

One hundred grams of the untreated peat were diluted with 300 ml. of distilled water. To this soil-water suspension 2.2 gm. of powdered $Ca(OH)_2$ were added, and the resulting mixture was stirred continuously for 12 hours. At the end of this time the pH of the suspension was taken, using a Beckman pH-meter, and was 6.15. The corresponding base saturation at this pH was 55 percent.

The soil-water mixture was placed on a Büchner funnel and filtered using suction. The soil was then washed several times with distilled water. After this the soil was air-dried and stored for future use.

COLUMN PACKING

To 10 gm. of the air-dried peat soil 40 ml. of 95-percent ethyl alcohol were added. The mixture was boiled under reduced pressure so as to remove air from the peat.

A short chromatographic column (13 mm. × 460 mm.) was then filled with the peat-alcohol suspension, applying 10 pounds of air pressure. On top of the level peat, a layer of 0.2 gm. of cellulose powder was placed. This cellulose powder was Whatman chromatography-grade ashless cellulose powder.

After the column was packed it was washed with distilled water to rid it of alcohol. Finally, the column was eluted, using a 0.001-M solution of $CaCl_2$, the concentration most likely to occur in the soil solution.

APPARATUS

An automatic drop-count fraction collector was used for taking the different fractions that were to be analyzed. With it small fractions of the liquid passing through the column were collected in a large number of test tubes fixed in a horizontal wheel. This wheel rotated sufficiently to move a new test tube under the outlet of the column as soon as a predetermined number of drops have been collected.

FRONTAL ANALYSIS

The behavior of a substance which travels through a chromatographic column in a given medium depends upon the affinity of the substance for the column material in the medium. If we allow a solution containing one component to pass through a column packed with an adsorbing medium, we can determine by analysis the position and concentration of that component in the effluent liquid. This is frontal analysis.

OPERATION

After the column was eluted for a reasonable time with the 0.001-M $CaCl_2$ solution it was removed from its position. The liquid on top of the

cellulose layer was removed carefully and, without allowing any air to enter the column, a 300 γP/ml. phosphate solution was placed in a fixed vertical position on top of the automatic fraction collector and the column liquid was displaced at the rate of 3 ml./hr. The flow of liquid was maintained constant by the use of a Mariotte bottle filled with the phosphate solution on top of the column and this, in turn, was connected to the column headpiece by means of Tygon tubing.

Fractions of 16 drops, or approximately 1 ml., were collected in 18 × 150-mm. test tubes and diluted to 3 ml. with distilled water before analyzing for phosphorus.

The exact weight of liquid that was analyzed was determined by weighing five empty test tubes and then reweighing them after the 16 drops were collected so as to obtain the volume of liquid per tube.

METHOD OF ANALYSIS

A modified procedure of the method developed by Kitson and Mellon (*9*) for the determination of phosphorus as molybdivanadophosphoric acid was used.

In the procedure 1-ml. aliquots of the solutions to be analyzed were taken. The aliquots were diluted with 1 ml. of distilled water and to each of them 1 ml. of 2.5-N HNO_3 was added. One milliliter of 0.125-percent ammonium vanadate and 1 ml. of 2.5-percent ammonium molybdate were then added. The solution was mixed well after each addition. Optical densities were read after 15 minutes at 470 μ using a Junior Coleman Spectrophotometer.

REAGENTS

Ammonium molybdate, 2.5 percent: Dissolve 12.5 gm. of $(NH_4)_7Mo_7O_{24}\cdot 4H_2O$ in distilled water and dilute to 500 ml.

Ammonium vanadate, 0.12 percent: Dissolve 1.25 gm. of NH_4VO_3 in 500 ml. of boiling water (use a 1-l. Pyrex volumetric flask), cool the solution somewhat, add 20 ml. of concentrated HNO_3, cool and dilute to 1 l.

Phosphate solution 300 γP/ml.: Dissolve 2.6409 gm. of KH_2PO_4 in 0.001-M $CaCl_2$ solution. Dilute to a volume of 2 l. with additional 0.001-M $CaCl_2$ solution.

DISCUSSION OF RESULTS

PHOSPHATE RETENTION BY UNTREATED PEAT

Triplicate runs were made for the studies on phosphate retention by the untreated peat. Values for phosphorus concentration expressed as γP/ml., are given for each tube analyzed in each run. See table 1.

TABLE 1.—*Frontal-analysis chromatography data for the untreated peat as* $\gamma P/ml$.

Tube No.	Replication 1	Replication 2	Replication 3
2	7		4
5	7		4
10	7	7	4
15	13	7	4
20	13	7	4
25	13	7	4
27	13	7	4
28	13	7	4
29	13	7	18
30	13	7	68
31	34	7	117
32	88	7	157
33	133	18	178
34	162	58	196
35	184	113	209
36	203	144	221
37	214	178	227
38	227	191	227
39	239	203	232
40	245	221	239
41	245	227	245
42	245	239	245
43	250	239	250
44	250	245	250
45	257	250	250
46	263	257	257
47	263	257	257
48	263	257	257
49	263	257	257
50	270	257	263
51	270	263	263
52	270	263	270
53	270	263	270
54	270	270	270
55	270	270	270
56	277	277	270
57	277	277	270
58	277	283	270
59	277	283	270
60	283	283	270
61	283	283	270
62	283	283	270
63	283	283	270
64	283	283	270
65	283	283	277
66	283	283	277
67	292	292	277
68	292	300	277
69	292	300	277

TABLE 1.—(*Continued*)

Tube No.	Replication 1	Replication 2	Replication 3
70	292	300	277
71	300	300	277
72	300	300	277
73	300	300	277
74	300	300	277
75	300	300	277
76	300	300	277
77		300	277
78		300	277
79		300	277
80			277
81			277
82			277
83			277
84			277
85			277
86			277
87			277
88			277
89			277
90			283
91			283
92			292
93			292
94			292
95			300
96			300
97			300
98			300
99			300
100			300

The curve shown in figure 1 represents a typical curve obtained by plotting optical density against fraction number. Data essential for the calculation of amount of phosphorus retained are given in table 2.

The mean amount of phosphorus retained by the acid peat was 636 ± 3.5 pounds per acre. Table 3 shows a summary of the amount of phosphorus retained by the untreated peat.

PHOSPHATE RETENTION BY PEAT TREATED WITH $Ca(OH)_2$

An increase in phosphorus retention was observed when the peat was treated with $Ca(OH)_2$. Data necessary for calculating the amount of phosphorus retained by the peat are given in table 4. Results of analyses on the

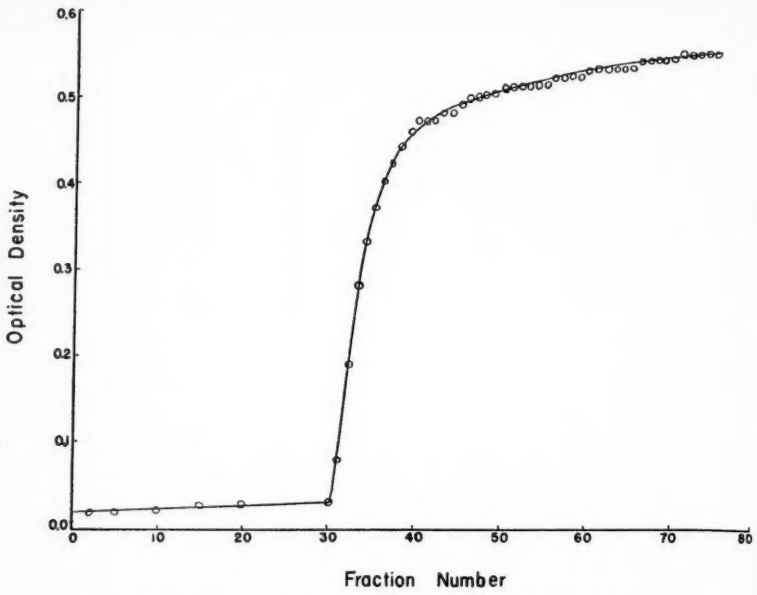

FIG. 1.—Optical densities of samples obtained in the study on phosphorus retention by an acid peat.

TABLE 2.—*Data for calculating the phosphorus retained by untreated peat*

Item		Run No. 1	Run No. 2	Run No. 3
Weight of peat	grams	10	10	10
Weight of cellulose	do.	.2	.2	.2
Weight of empty column	do.	49.96	49.96	49.22
Weight of column + peat + cellulose + liquid	do.	82.0	83.18	79.88
Fraction volume	do.	.981	.949	.998
Vo = column volume	do.	21.8	23.02	20.5
V_2	do.	32.3	33.7	31.1
V_2 − Vo, volume of solution from which P was removed	do.	10.5	10.7	10.6
Phosphorus concentration	γP/ml.	300	300	300
Amount of P retained	lb./A.	630	642	636

different fractions are seen in table 5. A typical curve obtained by plotting optical density versus fraction number is shown in figure 2.

When the untreated peat was eluted with the phosphate solution a point was reached at which the analyses of the fractions were the same as those

for the original solution. This point was never reached for peat treated with $Ca(OH)_2$. The shape of the curve obtained indicates that a very slow process by which phosphorus had been retained by the peat was occurring in the column behind the phosphate front. The phosphorus retained by the peat treated with $Ca(OH)_2$ was 1,094 ± 37.7 pounds of P/A. of soil. Table 6 shows a summary of the amount of phosphorus retained by the peat treated with $Ca(OH)_2$.

TABLE 3.—*A summary of the quantity of phosphorus retained (lb./A.) by the untreated peat*

Replication run No.	P retained	Mean	Standard deviation
1	630	—	—
2	642	636	±3.5
3	636	—	—

TABLE 4.—*Data for calculating the phosphorus retained by peat treated with $Ca(OH)_2$*

Item		Run No. 1	Run No. 2	Run No. 3
Weight of peat	grams	10	10	10
Weight of cellulose	do.	.2	.2	.2
Weight of empty column	do.	50.0	49.22	50.0
Weight of column + peat + cellulose + liquid	do.	82.51	81.42	82.76
Fraction volume	do.	.952	.974	.982
Vo = column volume	do.	22.3	22.0	22.6
V_2	do.	40.7	41.6	39.3
V_2 − Vo, volume of solution from which P was removed	do.	18.4	19.6	16.7
Phosphorus concentration	γP/ml.	300	300	300
Amount of P retained	lb./A.	1,104	1,176	1,002

As the treated peat was 55-percent base-saturated, but only 26-percent base-saturated when untreated, this means that an increase of 29 percent in base saturation caused a net increase of 458 pounds of phosphorus retained by the peat. In other words, the calcium treatment was responsible for the retention of that amount of phosphorus.

The increase in retained phosphorus possibly could be ascribed to three different forms of calcium phosphates in the peat: Tricalcium phosphate, dicalcium phosphate, and monocalcium phosphate. These forms of phosphates have different solubilities and, to a great extent, their presence is determined by the pH of the soils in which they occur. Tricalcium phos-

TABLE 5.—*Frontal-analysis chromatography data for peat treated with $Ca(OH)_2$ as $\gamma P/ml$.*

Tube No.	Replication 1	Replication 2	Replication 3
2	2	4	4
5	2	4	4
10	2	4	4
15	2	4	4
20	2	4	4
25	2	4	4
26	2	4	4
27	2	4	4
28	2	4	4
29	2	4	4
30	2	4	4
31	2	4	4
32	2	4	4
33	2	4	4
34	2	4	4
35	2	4	4
36	2	4	4
37	2	4	4
38	2	4	4
39	2	4	54
40	22	4	117
41	68	25	144
42	113	68	167
43	117	117	184
44	139	133	191
45	154	149	203
46	162	157	209
47	167	162	214
48	178	184	214
49	178	196	214
50	191	196	214
51	191	196	214
52	191	232	221
53	203	232	227
54	203	232	221
55	203	239	232
56	214	239	232
57	221	239	227
58	221	239	227
59	227	239	232
60	227	239	232
61	227	239	232
62	227	239	232
63	227	239	232
64	239	239	232
65	239	239	239
66	239	239	239
67	239	239	245
68	239	239	245
69	239	239	245

TABLE 5.—(*Continued*)

Tube No.	Replication 1	Replication 2	Replication 3
70	239	245	250
71	245	245	245
72	245	250	250
73	250	250	245
74	250	250	250
75	250	250	250
76	257	250	250
77	257	250	263
78	257	250	245
79	257	257	257
80	257	257	263
81	257	257	257
82	263	257	263
83	270	257	263
84	270	257	263
85	270	257	263
86	270	257	263
87	270	257	263
88	270	257	263
89	270	257	263
90	270	257	263
91	270	257	263
92	270	257	263
93	270	257	263
94	270	257	263
95	270	257	263
96	270	257	263
97	270	257	263
98	270	257	263
99	270	257	263
100		257	263
101			263
102			270
103			263
104			270
105			270
106			270
107			270
108			270
109			270
110			270
111			277
112			277
113			270
114			270
115			270
116			270
117			270
118			270
119			270
120			270

phate would be the least available of the three forms present, followed by the dicalcium phosphate and the monocalcium phosphate.

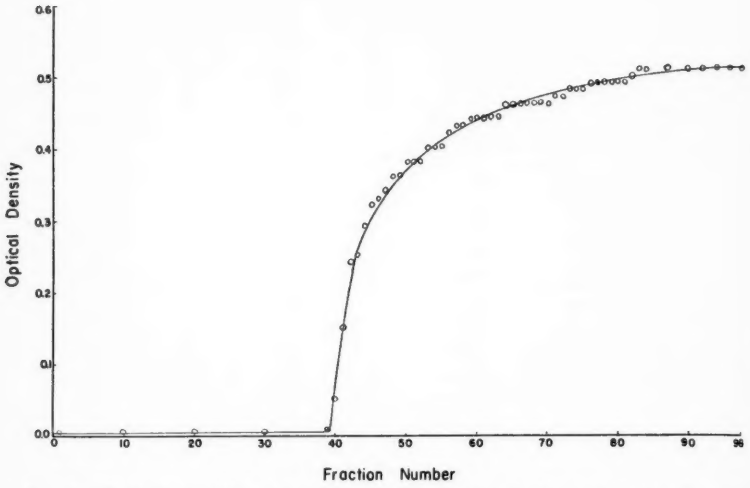

FIG. 2.—Optical densities of samples obtained in the study on phosphorus retention by a peat treated with $Ca(OH)_2$.

TABLE 6.—*A summary of the quantity of phosphorus retained (lb./A.) by peat treated with* $Ca(OH)_2$

Replication run No.	P retained	Mean	Standard deviation
1	1,104	—	—
2	1,176	1,094	±37.7
3	1,002	—	—

SUMMARY AND CONCLUSIONS

A refined procedure for the determination of phosphorus retention by an organic soil by means of frontal-analysis chromatography has been used with success.

The following conclusions were drawn from the investigations:

1. Frontal-analysis chromatography can be used for the determination of phosphate fixation in soils.

2. Calcium has a role in phosphate fixation in organic as well as in mineral soils.

RESUMEN

Se ha usado con éxito el análisis cromatográfico frontal para estimar la cantidad de fósforo retenido por un suelo orgánico.

Como resultado de esta investigación se ha llegado a la siguiente conclusión:

1. El análisis cromatográfico frontal puede usarse en investigaciones sobre la fijación de fósforo en suelos orgánicos.

2. En los suelos orgánicos, igual que en los suelos minerales, el calcio juega un importante papel en la fijación del fósforo.

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Factors that Govern the Sensitivity of Oat Seed to X-rays[1]

C. L. González-Molina[2]

INTRODUCTION

The sensitivity of crop plant seed to high dosages of X-rays varies with many factors, *e.g.*, seed size, crop variety, moisture content, etc. Sensitivity of seed to X-ray treatment is usually measured by loss of seed viability or a reduction in the vigor of X_1 seedlings.

Caldecott (*1*)[3] found a decrease in the sensitivity of barley seed when moisture content was raised from 4 to 8 percent, but there was little change over the range from 8 to 16 percent. Abrams (*2*) reported that oat seed containing 20 percent of moisture, and treated with 30,000 r of X-ray, produced a lower germination percentage and seedling vigor than seed treated with a 40,000-r dosage at 14 percent of moisture. He also concluded that oat varieties produced under similar environmental conditions differed in their sensitivity to X-rays.

Fröier and Gustafsson (*3*) treated Kolben and Velvet wheat varieties with 10,000 and 20,000 r and found large seed were affected less by X-rays than were small ones. The same authors, working with hulled and dehulled barley, and oat seed at dosages of 10,000 and 20,000 r of X-rays, discovered that the hull covering caused the seed to be less susceptible to radiation effects.

OBJECTIVE

The purpose of this study was to determine the relationship between certain physical characters of oat seed and their X-ray sensitivity, which, in turn, might elucidate varietal sensitivity differences.

The specific factors investigated were:

1. The effect of a hull covering over the oat caryopsis upon damage from X-rays.

[1] Thesis submitted to the Graduate Faculty of the Iowa State College, Ames, Iowa, in partial fulfillment of the requirements for the degree of Master of Science, August 1957.

[2] Research Assistant in Agronomy, Agricultural Experiment Station, University of Puerto Rico, Río Piedras, P.R. Appreciation is expressed to K. J. Frey for guidance throughout the course of this study; to J. W. Gowen and J. Stadler for radiating the experimental materials; to P. G. Homeyer and T. W. Hormer for suggestions on the statistical treatment of the data; and to L. Everson who made the plant-germinator facilities available. All are on the staff of the Iowa State College, Ames, Iowa.

[3] Italic numbers in parentheses refer to Literature cited p. 211.

2. The relative sensitivity of large and small seed of the same oat variety to X-rays.

3. The relative damage done to the radicals and plumules from treated seed.

MATERIALS AND METHODS

For ease of presentation the different experiments will be referred to by numbers throughout this paper. In experiment I, three oat varieties, Mo. 0-205, Simcoe, and Bonham, produced under the same environmental conditions, were used to study the relationship between the hull covering and X-ray sensitivity of seed. To eliminate any confusing effects due to differential seed weights between varieties, seed of equal size were selected from each variety. The seed weights for both hulled and dehulled samples are given in table 1. The dehulling was done by hand.

TABLE 1.—*Weights (grams) of 1,500 of hulled and 1,500 dehulled seed of 3 oat varieties used*

Variety	Hulled-seed weight	Dehulled-seed weight
Mo. 0-205	45.4	—
	45.5	33.5
Simcoe	45.4	—
	45.6	34.0
Bonham	45.5	—
	45.6	33.2

The seed were adjusted to moisture percentages of 13.8 and 20.2 by holding them for 2 weeks in desiccators over a saturated sodium chloride solution and distilled water, respectively. Seed containing approximately 14 percent of moisture were treated with 40,000 r of X-ray, while the seed of about 20-percent moisture were given a 30,000-r dosage.

The seed were radiated with a G.E. Maxitron X-ray machine operated at 250 kvp. and 30 ma. with the radiation filtered through a 0.25-mm. Cu + Al filter. The distance from the anode to the target was 16.5 cm. For radiation the oat seed were placed in a plastic container measuring 3 inches in diameter and divided into four triangular compartments; thus, four different seed lots could be radiated simultaneously.

Within 24 hours after being radiated the seed were planted in the greenhouse in flats containing a sterilized soil mixture of loam, peat, and sand in the ratio of 2:1:1. The planting depth was 1 inch. A plot consisted of 100 seed planted in a row across a flat, and from 6 to 10 rows were sown per flat. The experimental design was a randomized block with three replications. Hulled and dehulled nonradiated seed containing approxi-

mately 8.5 percent of moisture were included as checks for each variety. Germination percentages and seedling heights, in centimeters, were determined 21 days after planting. On the same day the seedlings were cut at the soil surface, and subsequently dried at 30°C. and weighed. Dry weights were adjusted to weight per 100 seedlings, in grams.

The second factor investigated was the relationship between seed and germ size and X-ray sensitivity. Removal of the germs from oat seed proved to be difficult, but a preliminary test of treating the oat seed in solutions of H_2SO_4 (0.4768N) and water at 68°C. for varying intervals, showed the most satisfactory treatment was at a concentration of 1 part H_2SO_4 to 25 parts of water exposed in the oven for 1 hour. The germ and endosperm data from large and small seed of Bonham variety are given in table 2. The exposed area of germ surface was 281 and 227 square units for large and small seed, respectively.

TABLE 2.—*Weights (grams) of germ and endosperm in large and small seed of Bonham oat variety*

Item	Large seed	Small seed
Weight per 100 dehulled seed	3.94	2.53
Dry weight of germ	.16	.12
Dry weight of endosperm	2.52	1.67
Percentage of germs in seed	6.35	7.19

To determine whether seed size *per se* was responsible for differences in X-ray sensitivity, the endosperm of the large seed in experiment II were trimmed off until the seed weights were the same as those of the small seed. All of the seed for this and subsequent experiments were dehulled before being tempered to 14-percent moisture. For this experiment the seed were treated with 25,000 r of X-rays. The different seed lots, large seed, trimmed and untrimmed, and small seed, each treated and untreated with X-rays, were planted and assayed in the greenhouse using the same techniques as those in experiment I.

When the plastic container was used in radiating the seed it was improbable that all in a compartment received an equal X-ray dosage. Therefore, experiment III was conducted in which, before the radiation treatment, the seed were placed with the germ upward, alternating large, small, large, etc., in a single layer around the circumference of a circle. The center of the circle coincided with the center of the X-ray target area so that the X-rays were uniformly distributed over the seed. The proposed X-ray dosage for this experiment was 25,000-r units[4].

[4] A dosage lower than 25,000 r was probable from the germination data.

The radiated seed with appropriate checks were planted and the experiment assayed in the same manner as the earlier experiments.

Experiment IV, using a dosage of 35,000 r, was also conducted to compare the X-ray sensitivity of large and small seed of the Bonham variety. The radiation was carried out in the plastic container in the same manner as described previously, except that every 10 minutes during the radiation period the container was shaken to change the position of the seed, and thus give a more equal chance of uniform dosage to all.

The treated seed from this experiment were divided in two. The radiated seed in the first group were planted in the greenhouse following the technique of previous experiments. The other seed were germinated on blotters in a plant germinator under constant moisture, temperature, and light conditions. A plot consisted of 50 seed uniformly spaced on a blotter, and the experimental design was a randomized block with three replications. After 6 days measurements were taken on germination percentage and length of stem and center root. In addition, the stems and the center roots of five seedlings in each plot were measured daily to determine the rate of development of each.

Finally, in experiment V, large and small seed of the Bonham oat variety were treated with 30,000 and 40,000 r of X-ray. The seed were stuck in alternating rows of large and small on a flat cardboard surface measuring 12 x 16 cm. The distance from the anode to the target was 32.5 cm., which resulted in a center portion of the target, measuring 8 x 8 cm., receiving a higher dosage than the surrounding area.

Therefore, the cardboard was so cut that the center portion could be removed when the seed on it had received the proper dosage. The surrounding area remained under the machine until it had received a dosage equivalent to the center portion. The radiated material was sown within 24 hours in both the greenhouse and plant propagator, using the technique described for previous experiments.

EXPERIMENTAL RESULTS

HULLED *VS.* DEHULLED SEED

The germination percentages, average seedling heights, and weights per 100 seedlings of three oat varieties grown in experiment I are presented in tables 3 to 5, respectively. The pertinent mean squares from the analyses of variance of these data are given in table 6 for the two X-ray dosages. As expected, the X-ray treatment reduced the germination percentages of both hulled and dehulled seed materially. However, as can be noted, there was a significant interaction between varieties and hulled *vs.* dehulled seed at the 40,000-r treatment. There is no apparent explanation for the

TABLE 3.—*Germination percentages of hulled and dehulled oat seed of 3 varieties when radiated and not radiated in experiment I*

Treatment and condition of seed	Mo. 0–205			Simcoe			Bonham		
	Radiated	Non-radiated	Check[1]	Radiated	Non-radiated	Check[1]	Radiated	Non-radiated	Check[1]
40,000 r of X-ray									
Hulled	32.6	99.3	99.3	63.6	98.6	99.0	9.3	99.0	98.3
Dehulled	3.3	98.6	99.3	68.3	97.3	98.0	39.6	99.0	99.0
30,000 r of X-ray									
Hulled	20.3	94.0	99.0	49.0	75.0	98.6	54.6	75.3	98.0
Dehulled	1.0	61.0	—[2]	17.6	52.6	—[2]	4.0	46.6	—[2]

[1] Seed contained 8.5 percent of moisture.
[2] No dehulled check included.

TABLE 4.—*Height of seedlings (centimeters) from hulled and dehulled oat seed of 3 varieties when radiated and not radiated in experiment I*

Treatment and condition of seed	Mo. 0–205			Simcoe			Bonham		
	Radiated	Non-radiated	Check[1]	Radiated	Non-radiated	Check[1]	Radiated	Non-radiated	Check[1]
40,000 r of X-ray									
Hulled	9.2	28.9	27.2	9.1	30.0	30.4	3.7	26.1	26.0
Dehulled	2.4	28.0	27.0	6.2	28.7	28.0	7.0	25.9	26.1
30,000 r of X-ray									
Hulled	8.2	25.1	26.6	7.0	28.6	29.6	5.3	25.5	26.6
Dehulled	1.0	26.9	—[2]	8.5	28.0	—[2]	2.4	25.2	—[2]

[1] Seed contained 8.5 percent of moisture.
[2] No dehulled check included.

TABLE 5.—*Dry weight per 100 seedlings (grams) from hulled and dehulled oat seed of 3 varieties when radiated and not radiated in experiment I*

Treatment and condition of seed	Mo. 0–205			Simcoe			Bonham		
	Radiated	Non-radiated	Check[1]	Radiated	Non-radiated	Check[1]	Radiated	Non-radiated	Check[1]
40,000 r of X-ray									
Hulled	0.542	1.742	1.706	0.388	1.831	1.945	0.332	1.726	1.643
Dehulled	.108	1.769	1.465	.410	1.577	1.364	.442	1.613	1.602
30,000 r of X-ray									
Hulled	.412	1.704	1.574	.916	1.273	1.660	.381	1.830	1.720
Dehulled	.033	2.057	—[2]	.942	1.094	—[2]	.368	1.799	—[2]

[1] Seed contained 8.5 percent of moisture.
[2] No dehulled check included.

TABLE 6.—*Mean squares from analyses of variance of germination percentages, seedling heights, and dry weights per 100 seedlings of 3 oat varieties from seed radiated in experiment I*

Source of variation	Degrees of freedom	Mean squares[1]		
		Germination	Seedling height	Dry weight
40,000 r of X-ray				
Treatments *vs.* checks	1	11,844**	1,278.7**	3.933**
Within treatments	11	4,182**	397.8**	1.476**
Variety	2	1,946**	24.0**	.008
Hulled *vs.* dehulled	1	3	17.4*	.138*
Radiation	1	35,156**	4,221.1**	15.748**
Variety × hulled *vs.* dehulled	2	685**	22.6**	.045
Variety × radiation	2	2,125**	2.9	.035
Hulled *vs.* dehulled × radiation	1	15	4.3	.016
Variety × hulled *vs.* dehulled × radiation	2	659**	16.8**	.085
Within checks	5	0	8.0*	.122**
Variety	2	1	15.6**	.007
Hulled *vs.* dehulled	1	0	3.2	3.372**
Variety × hulled *vs.* dehulled	2	1	2.9	.112*
Error	34	8	2.6	.033
30,000 r X-ray				
Treatments *vs.* checks	1	19,971**	992.2**	2.453**
Among treatments	11	2,637**	388.4**	1.375**
Variety	2	70*	40.5**	.007
Hulled *vs.* dehulled	1	8,949**	17.6*	.012
Radiation	1	16,727**	4,074.7**	11.242**
Variety × hulled *vs.* dehulled	2	169**	8.8	.004
Variety × radiation	2	1,303**	1.1	1.713**
Hulled *vs.* dehulled × radiation	1	81*	25.7**	.066
Variety × hulled *vs.* dehulled × radiation	2	235**	26.6**	.183**
Among checks	2	1	9.0	.016
Error	28	16	3.1	.028

[1] * Denotes significance at 5-percent level; ** denotes significance at 1-percent level.

reversal in germination percentages among varieties due to the presence or absence of hulls covering the seed.

At the 30,000-r dosage the results were in line with expectation. In all three varieties the seed with a hull-covering had a higher germination percentage than those without. Apparently the tempering process of using water in the dessicator also had an effect upon the germination percentage. Nevertheless, the germination reductions due to radiation were much greater in the dehulled than in the hulled seed lots, as is shown by the highly significant interaction mean square for "hulled *vs.* dehulled × radiation" (table 6). Data from this portion of experiment I indicate that a hull-covering over the seed has some protective effect against X-ray damage.

Seedling heights from seed receiving the 40,000-r dosage of X-rays showed a variety × radiation interaction similar to that in germination. The interaction between varieties and hulled *vs.* dehulled was highly significant.

With the 30,000-r treatment the heights of seedlings from dehulled seed were reduced more by radiation than were those from hulled seed in Mo. 0-205 and Bonham varieties, but not in Simcoe. This reaction is apparent in the highly significant mean square for the second-order interaction. Dehulling of the seed seemed to have a slight but consistent effect of reducing the seedling heights of nonradiated checks. The only exception was Mo. 0-205 at the 20-percent moisture level.

Another measure of seedling vigor was weight per 100 seedlings. The effects of the hulls on X-ray sensitivity as measured by weights of the seedling were not consistent. For Mo. 0-205 the seedling weights were much less for dehulled than for hulled seed under both the 30,000- and 40,000-r treatments. Simcoe seedlings were heavier from the dehulled seed in both cases, and Bonham seedlings from the dehulled seed were heavier under the 40,000-r than at the 30,000-r treatment.

There was no consistent effect of the hull-covering on the X-ray sensitivity when measured in terms of X_1 seedling vigor. In some cases the dehulling resulted in smaller seedlings after radiation and, in others, larger. In general, seedlings from nonradiated, dehulled seed were slightly, but consistently, smaller than those from seed with hulls intact. This is indicated by a highly significant mean square for "hulled *vs.* dehulled" within checks for weights per 100 seedlings.

In general, the results of experiment I were rather inconclusive relative to the effect of a hull-covering on the X-ray sensitivity of oat seed. Dehulling oat seed *per se* did not decrease the germination percentage if the seed were maintained at approximately 14 percent of moisture, but at 20-percent moisture the dehulled seed were reduced much more in viability

than were seed with hull intact. Dehulling also seemed to cause a slight but consistent reduction in seedling vigor in the varieties Simcoe and Bonham.

LARGE *VS.* SMALL SEED

The germination percentages, seedling heights, and weights per hundred seedlings for the large- and small-seed comparisons in the greenhouse grown

TABLE 7.—*Germination percentages, seedling heights, and dry weights per 100 seedlings from trimmed and untrimmed seed of Bonham oat variety when radiated and not radiated in experiment II*

Kind and condition of seed	Germination (percent)			Seedling heights (percent of check)			Dry weight per 100 seedlings (percent of check)		
	Radiated	Non-radiated	Check[1]	Radiated	Non-radiated	Check[1]	Radiated	Non-radiated	Check[1]
Large seed with trimmed endosperm	71	85	—	39	67	—	15	29	—
Large seed with untrimmed endosperm	84	96	96	68	107	100	43	106	100
Small seed	90	97	99	61	100	100	48	91	100

[1] Seed contained 8.5 percent of moisture.

TABLE 8.—*Mean squares associated with seedling heights and weights per 100 seedlings of checks from seed of Bonham oat variety in experiment II*

Source of error	Degrees of freedom	Mean squares[1]	
		Height	Dry weight per 100 seedlings
		Cm.	*Gm.*
Check *vs.* treatment	1	73.0	0.901
Large *vs.* small checks	1	8.3*	.192*
Error	14	1.8	.022

[1] * Denotes significance at 5-percent level.

portions of experiments II, III, IV, and V are given in tables 7 to 14. Pertinent mean squares from analyses of variance of the data from these experiments are presented in tables 9, 11, 13, and 15, respectively. In experiment II trimming endosperm from the large seed reduced their germination to 85.0 percent, a highly significant reduction. Presumably a more rapid absorption of water and distintegration of compounds in the seed caused the deterioration in seed viability. After this experience endosperm trimming was eliminated as a treatment in subsequent experiments.

In none of the four experiments was there a significant difference in germination percentage between nonradiated large and small seed, regardless of whether the moisture content of the seed was tempered to 14.0 percent or maintained at 8.5. When the seed were radiated and planted

TABLE 9.—*Mean squares from analyses of variance of germination percentages, seedling heights, and dry weights per 100 seedlings from seed of Bonham oat variety, as percentage of checks from experiment II*

Source of variation	Degrees of freedom	Mean squares[1]		
		Germination	Seedling height	Dry weight
Among treatments[2]	7	256**	19**	40**
Moisture level	1	460**	31**	90**
Between checks	1	11	0	0
Cut *vs.* noncut	1	432**	37**	76**
Within cuttings	1	280**	12**	3
Seed size	1	30*	1	0
Radiation	1	252**	46**	84**
Seed size × radiation	1	19	0	3
Error	14	4	1	2

[1] * Denotes significance at 5-percent level; ** denotes significance at 1-percent level.

[2] Nonorthogonal breakdown.

TABLE 10.—*Germination percentages, seedling heights, and dry weights per 100 seedlings from large and small oat seed of Bonham variety when radiated and not radiated in experiment III*

Seed size	Germination (percent)			Seedling heights (percent of check)			Dry weight per 100 seedlings (percent of check)		
	Radiated	Non-radiated	Check[1]	Radiated	Non-radiated	Check[1]	Radiated	Non-radiated	Check[1]
Large	92	99	97	63	106	100	58	108	100
Small	97	99	99	65	103	100	62	92	100

[1] Seed contained 8.5 percent of moisture.

in the greenhouse, the percentage of seedlings emerging from the soil after 21 days, referred to herein as "germination percentage", was lower for the large than for the small seed. For dosages from 25,000 to 35,000 r this difference was small and significant in only one case, but at the 40,000-r dosage the germination percentages were 94.0 and 66.0 for the small and large seed, respectively. This difference was highly significant. These data are contrary to those of Fröier and Gustafsson (*3*), but it is suggested that

TABLE 11.—*Mean squares from analyses of variance of germination percentages, seedling heights, and dry weights per 100 seedlings from seed of Bonham oat variety, as percentage of the checks in experiment III*

Source of variation	Degrees of freedom	Mean squares[1]		
		Germination	Seedling height	Dry weight
Treatment *vs.* checks	1	3	9.6**	15.6*
Among treatments	3	39**	16.3**	17.2*
Seed size	1	21**	.1	1.1
Radiation	1	75**	48.6**	47.5**
Seed size × radiation	1	21**	0	3.2
Large check *vs.* small check	1	6	0	0
Error	10	2	.4	3

[1] * Denotes significance at 5-percent level; ** denotes significance at 1-percent level.

TABLE 12.—*Germination percentages, seedling heights, and dry weights per 100 seedlings from large and small seed of Bonham oat variety when radiated and not radiated in experiment IV*

Seed size	Germination (percent)			Seedling height (percent of check)			Dry weight per 100 seedlings (percent of check)		
	Radiated	Non-radiated	Check[1]	Radiated	Non-radiated	Check[1]	Radiated	Non-radiated	Check[1]
Large	87	98	99	46	92	100	34	112	100
Small	96	99	98	68	98	100	55	99	100

[1] Seed contained 8.5 percent of moisture.

TABLE 13.—*Mean squares from analyses of variance of germination percentages, seedling heights, and dry weights per 100 seedlings from seed of Bonham oat variety as percentage of checks from greenhouse-grown portion of experiment IV*

Source of variation	Degrees of freedom	Mean squares[1]		
		Germination	Seedling height	Dry weight per 100 seedlings
Checks *vs.* treatment	1	40	24*	25**
Among treatments	3	87**	17*	41**
Seed size	1	75*	5	1
Radiation	1	147**	45**	113**
Seed size × radiation	1	40	2	9*
Large check *vs.* small check	1	1	0	0
Error	10	10	3	1.4

[1] * Denotes significance at 5-percent level; ** denotes significance at 1-percent level.

their results might have been different because they used another X-ray dosage.

When the data on seedling heights and weights per seedling were analyzed for experiments II through V it was found that the seedlings

TABLE 14.—*Germination percentages, seedling heights, and dry weights per 100 seedlings from large and small oat seed of Bonham variety when radiated and not radiated in experiment V*

Seed size	Germination (percent)			Seedling heights (percent of check)			Dry weight per 100 seedlings (percent of check)		
	Radiated at—		Non-radiated	Radiated at—		Non-radiated	Radiated at—		Non-radiated
	30,000 r	40,000 r		30,000 r	40,000 r		30,000 r	40,000 r	
Large	95	66	96	64	45	100	72	28	100
Small	96	94	100	75	64	100	68	54	100

TABLE 15.—*Mean squares from analyses of variance of germination percentages, seedling heights, and dry weights per 100 seedlings from seed of Bonham oat variety, as percentage of checks from the greenhouse-grown portion of experiment V*

Source of variation	Degrees of freedom	Mean squares[1]		
		Germination (percent)	Seedling height (percent of check)	Dry weight per seedlings (percent of check)
Among treatments	5	450**	14**	23**
Checks *vs.* radiation	1	400**	57**	71**
Large check *vs.* small check	1	24	0	0
30,000 r *vs.* 40,000 r	1	705**	7**	34**
Large 30,000 r *vs.* small 30,000 r	1	1	2**	0
Large 40,000 r *vs.* small 40,000 r	1	1,121**	5**	10**
Error	10	21	.10	.43

[1] * Denotes significance at 5-percent level; ** denotes significance at 1-percent level.

from nonradiated large seed were always significantly larger than those from small seed (*e.g.* table 8). Thus, any differences which existed between the seedlings from radiated large and small seed would undoubtedly be confounded with the initial differences between these two seed classes. To eliminate this confounding factor the seedling heights and weights per 100 seedlings of the large and small checks with 8.5 moisture were regarded as equal to 100, and the other data were expressed as a percentage of these

checks. All the data on seedling heights and weights per 100 seedlings in tables 7, 10, 12, and 15 are expressed in this way.

In experiments II and III where the X-ray dosages were 25,000 r or less, seedling heights and weights per 100 seedlings from radiated large and small seed were not significantly different when expressed relative to the checks. For example, the relative values for seedling heights from large and small seed were 68 and 61, respectively, in experiment II, and 63 and 65 in experiment III. Even at the 30,000-r dosage in experiment V the relative values for seedling heights and weights per 100 seedlings were not materially different between large and small seed. In experiment IV, wherein the X-ray dosage was 35,000 r, the relative values for seedling vigor were significantly lower for the seedlings from large than from small seed. The relative values for plant heights were 46 and 66, respectively, and for weights per 100

TABLE 16.—*Germination percentages and length of stems and center roots of plant-germinator-grown seedlings from large and small seed of Bonham oat variety when radiated and not radiated in experiment IV*

Seed size	Germination			Length of center root			Length of stem		
	Radiated	Non-radiated	Check[1]	Radiated	Non-radiated	Check[1]	Radiated	Non-radiated	Check[1]
	Percent	*Percent*	*Percent*	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*
Large	99	100	100	2.58	8.25	7.27	1.28	4.99	4.64
Small	99	100	100	2.44	6.98	7.64	1.10	4.26	5.44

[1] Seed contained 8.5 percent of moisture.

seedlings 34 and 55, respectively. The results from the 40,000-r treatment in experiment V were nearly identical with those from experiment IV. From these experiments it appears that the X_1 seedling vigor from large seed is relatively more damaged by X-ray treatment than that from small ones. The differences were small to nonexistent at a dosage of 25,000 r or less, but became greater as the dosage was increased up to 40,000 r. At the highest dosage the X_1 seedlings from large seed were only about two-thirds as vigorous as those from small seed.

RESULTS WITH SEED UNDER CONTROL IN PLANT GERMINATOR

A portion of the seed in experiments IV and V was sown in a plant germinator under controlled light, moisture, and temperature conditions. Data for germination percentages and length of stem and center roots for these experiments are given in tables 16 and 18. The pertinent mean squares from the analyses of variance are presented in tables 17 and 19. Radiation of the seed with 30,000, 35,000, or 40,000 r of X-ray had no effect on their germination, either large or small. Significant differences existed be-

TABLE 17.—*Mean squares from analyses of variance of germination percentages and lengths of stems and center roots from plant-germinator-grown Bonham oat variety seedlings in experiment IV*

Source of variation	Degrees of freedom	Mean squares[1]		
		Germination	Length of center roots	Length of stems
Check *vs.* treatments	1	1.0	22.88**	18.18**
Among treatments	3	1.2	26.92**	21.10**
Seed size	1	—	1.48	.63
Radiation	1	—	78.74**	35.43**
Seed size × radiation	1	—	.09	.23
Large check *vs.* small check	1	0	.05	.96*
Error	10	.5	.35	.15

[1] * Denotes significance at 5-percent level; ** denotes significance at 1-percent level.

TABLE 18.—*Germination percentages and length of stems and center roots of plant-germinator-grown seedlings from large and small seed of Bonham oat variety when radiated and not radiated in experiment V*

Seed size	Germination			Length of center root			Length of stem		
	30,000 r of X-ray	40,000 r of X-ray	Non-radiated	30,000 r of X-ray	40,000 r of X-ray	Non-radiated	30,000 r of X-ray	40,000 r of X-ray	Non-radiated
	Percent	*Percent*	*Percent*	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*
Large	100	99	99	2.4	1.3	9.3	2.5	1.7	9.1
Small	99	100	100	3.1	1.3	9.2	2.7	1.6	7.6

TABLE 19.—*Mean squares from analyses of variance of germination percentages and lengths of stems and center roots of plant-germinator-grown Bonham oat variety seedlings in experiment V*

Source of variation	Degrees of freedom	Mean squares[1]		
		Germination	Length of center roots	Length of stems
Among treatments	5	0.4	43.19**	32.05**
Check *vs.* radiation	1	0	208.95**	153.93**
Large checks *vs.* small checks	1	.7	.01	3.26*
30,000 r *vs.* 40,000 r	1	0	6.21**	3.00*
Large 30,000 r *vs.* small 30,000 r	1	.7	.76	.04
Large 40,000 r *vs.* small 40,000 r	1	.7	0	.01
Error	10	.8	.33	.50

[1] * Denotes significance at 5-percent level; ** denotes significance at 1-percent level.

tween the stem lengths from large and small check seed in both experiments, but the root lengths did not differ in either one.

Several seedlings in each plot in experiment V, sown in the germinator, were measured daily for length of stem and center root. Data from these observations are given in table 20. The center rootlet in the checks elongated faster than the stem during the first couple of days, but after that the stem

TABLE 20.—*Daily length (centimeters) of stems and center roots of plant-germinator-grown Bonham oat variety seedlings in experiment V*

Kind of seed and treatment	Stem on indicated days after planting				Center root on indicated days after planting			
	1	2	3	4	1	2	3	4
Large seed, 30,000 r of X-rays	0.2	0.6	1.0	2.5	0.8	1.4	2.0	2.4
Large seed, nonradiated	.5	1.6	3.0	9.1	1.3	2.9	5.4	9.3
Small seed, 30,000 r of X-rays	.3	.6	.9	2.5	.7	1.5	2.2	3.1
Small seed, nonradiated	.4	1.3	2.7	7.6	1.0	2.9	5.5	9.2
Large seed, 40,000 r of X-rays	.2	.4	.7	1.7	.6	1.0	1.3	1.3
Small seed, 40,000 r of X-rays	.2	.4	.7	1.6	.6	1.0	1.2	1.3

TABLE 21.—*Ratio of length (centimeters) of lateral to center rootlets of plant-germinator-grown Bonham oat variety seedlings in experiment V*

Kind of seed and treatment	Length of center root	Length of lateral roots	Ratio L/C
Large seed, 30,000 r of X-rays	2.4	1.47	0.61
Large seed, nonradiated	9.3	8.99	.97
Small seed, 30,000 r of X-rays	3.1	1.69	.55
Small seed, nonradiated	9.2	8.90	.97
Large seed, 40,000 r of X-rays	1.3	.60	.46
Small seed, 40,000 r of X-rays	1.3	.39	.30

tended to grow faster. Radiation at either a 30,000- or 40,000-r dosage reduced root and stem growth to about the same relative extent. In table 21 the ratios of lateral to central root lengths are shown. In the check seedlings from both large and small seed the ratio was 0.97, while in the radiated seedlings the ratios ranged from 0.30 to 0.61. This indicates that the lateral roots were affected much more by radiation than was the central one.

The data from the plant germinator suggest that the reduction in germination observed in greenhouse tests does not result from seeds being killed by X-rays, but simply from a reduction in vigor. When the seed were

planted 1 inch deep in the soil mixture many of the X-rayed ones were not vigorous enough to emerge from the soil.

DISCUSSION

The results reported herein show that radiated oat seed containing 14 percent of moisture were less susceptible to damage from X-ray treatment than those containing 20 percent. Tempering seed to 20 percent of moisture caused a decrease in seed viability *per se*. This may have been produced by an initiation of mitosis in the high-moisture seed, followed by lethality in certain seed when they were dried again.

The protection afforded seed embryos by a hull-covering varied with the moisture content of the seed and with variety. Varietal response to X-rays could possibly be due to the characteristics of the hulls of the different varieties. Thickness of the hull or interference with moisture absorption

TABLE 22.—*Ratios of relative vigor reduction of large to small Bonham oat seed caused by varying dosages of X-rays in experiments II to V*

Experiment No.	Dosage of X-ray (r)	Seedling height	Weights per 100 seedlings
II	25,000	107	80
III	25,000	90	71
IV	35,000	66	64
V	30,000	69	114
	40,000	65	64

into the seed could be factors where the hull influenced the X-ray sensitivity of oat seed. In this experiment dehulled seed absorbed moisture more quickly than their hulled counterparts. Under any circumstances the data from experiment I relative to the effect of hull-covering on the X-ray sensitivity of oat seed were rather inconclusive. The differential varietal reactions noted suggest that study of this factor could well be pursued further.

Since the embryo or germ of an oat seed is the portion which produces the young seedling, it might be reasoned that small seed would show less damage because the X-ray target area is smaller. The average weights of the germs from the large and small seed of Bonham variety were 0.16 and 0.12 gm., respectively, and the germ surface areas were 281 and 227 square units, respectively. The ratios of small to large seed for weight and surface were 0.75 and 0.80 respectively. The ratios of relative damage of large to small seed measured by reductions in plant height and weights per 1 hundred seedlings are given in table 22.

The ratio of relative damage for seedling heights appears to vary with

the dosage of X-ray. At the 25,000-r level the relative damage to both seed sizes was about the same, whereas at from 30,000 to 40,000 r the relative damage to the X_1 seedlings from small seed was only two-thirds as great as that to those from large ones. At the lower dosage the ratio of relative damage was higher than either the germ weight or surface-area ratios and at the higher dosages it was smaller.

For weights per 100 seedlings the ratio of relative damage between X_1 seedlings from large and small seed was in line with the ratios of germ weights and surface area. At 35,000- and 40,000-r levels the seedlings from small seed showed only about two-thirds as much damage as those from large seed. For some unexplainable reason the X_1 seedlings from large seed given the 30,000-r dose in experiment V were not damaged as much as those from small seed.

It appears that the differences in germ size between large and small oat seed do not account for all of the differential damage obtained in X_1 seedlings from radiation with high dosages of X-rays. At least the ratios of target weights and surface areas do not correspond too closely to the ratios of relative damage. Furthermore, the latter ratios tend to vary with dosage. Perhaps one would find a closer relationship between growing-point ratios and ratios of relative damage between small and large seed. Also, the differential ratios of relative damage with different X-ray dosages may be confounded with double hits.

A discrepancy was observed between the germination percentages of radiated seed when grown in the greenhouse and in the plant germinator. In the greenhouse the germination percentages were reduced by high X-ray dosages, while in the plant germinator even the seed lot receiving 40,000 r germinated 99 percent. In all probability the same percentage of seed sprouted in the greenhouse soil and on the blotters in the germinator, but in the first case the seedlings had to grow through 1 inch of soil before they were classed as germinated. Any seedlings which lacked the vigor to accomplish this would be classed as from dead. In the germinator any seed which sprouted were classed as germinated. This suggests that all of the reactions observed were due to reduced vigor of the seedlings and not to seed lethality. Very likely this vigor reduction is due to the effect of X-rays upon the chromosones which, in turn, is related to cell division. This also emphasizes the fact that seedling vigor is probably the best measure of damage done by X-rays.

SUMMARY

A series of experiments was performed designed to determine the relationship between hull covering and seed and germ size to X-ray sensitivity of oat seed of three varieties: Mo. 0-205, Simcoe, and Bonham. The seed

contained approximately 14 and 20 percent of moisture and X-ray dosages of 40,000 and 30,000 r were used. The data indicated that:

1. Hull-coverings may or may not afford protection to the seed from X-rays. Both varieties and moisture content produced differential reactions.

2. Large seed were more affected by high X-ray dosages than were small ones. Larger seed have larger germs, exposing more area to X-ray effects. A decrease of germination and seedling vigor was obtained when large seed were exposed to radiation, although there was no indication of a proportional decrease between germ size and relative damage to seedlings from large and small seed.

3. Germination percentages of oat-seed lots receiving up to 40,000 r of X-rays were as good as those of the checks, when measured on blotters in a plant germinator, but seedling vigor was materially reduced. Lowered germination percentages noted in greenhouse experiments were probably due to insufficient vigor to grow through the soil on the part of many seedlings. Probably all of the X-ray effects at the dosages used were caused by decreases in seedling vigor.

4. Radicals and plumules of the seed were equally damaged by X-ray treatment.

RESUMEN

En una serie de experimentos diseñados para determinar si la testa de la semilla de avena y el tamaño del embrión afectaban su sensibilidad a radiaciones de rayos X, se obtuvieron los siguientes resultados:

1. La testa puede o no proteger la semilla de los efectos de rayos X. Tanto la variedad como el contenido de humedad de la semilla afectan la naturaleza de la reacción.

2. Las semillas grandes fueron más afectadas por dosis altas de rayos X que las semillas pequeñas. Como las semillas de mayor tamaño poseen embriones más grandes, por eso exponen mayor área a los efectos de los rayos X. Una reducción en germinación y en vigor vegetativo fué obtenida cuando semillas de mayor tamaño fueron expuestas a radiación. No hubo indicación de reducción proporcional entre el tamaño del embrión y el daño ocasionado a las plantitas obtenidas de semillas grandes y pequeñas.

3. Bajos condiciones controladas en el laboratorio, el porcentaje de germinación de la semilla de avena que recibió hasta 40,000-r de rayos X fué igual al de los testigos. No obstante, el vigor vegetativo se redujo grandemente. La reducción en el porcentaje de germinación observado bajo condiciones de invernadero se debió probablemente al vigor insuficiente de muchas plantitas para crecer a través de la capa de suelo que cubría la semilla. Posiblemente el efecto de las diferentes dosis de rayos X usados produjo una reducción en el vigor vegetativo de las plantitas.

4. Las radículas y las plúmulas de las semillas fueron igualmente damnificadas por el tratamiento de rayos X.

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1. Caldecott, Richard S., Effects of hydration on X-ray sensitivity in Hordeum, *Radiation Res.* **3** 316–30, 1955.
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RESEARCH NOTE

VITAMIN C IN ACEROLA AND ROSE HIPS

In a valuable and beautifully illustrated publication by Bruce R. Ledin on the acerola,[1] the vitamin C content of different fruits, on the basis of ascorbic acid in milligrams per 100 gm. of juice or edible matter, are compared. According to the author, the figures reported in table 1 of his paper are taken from several publications and presented in descending order as to their ascorbic acid content, to compare them with the acerola. In this table Ledin rated *Rosa rugosa* hips first, because they contain 1,700 to 6,977 mg. of ascorbic acid per 100 gm. of edible matter, and assumed these values to be on wet basis, the only acceptable basis of comparison for the values quoted for the acerola. The Barbados cherry, as Ledin prefers to call the acerola, is rated second with values between 1,000 to 4,676 mg. of ascorbic acid per 100 gm. of juice taken from Asenjo and Guzmán[2] and Mustard[3] publications.

Although Ledin does not state the source of his information on *Rosa rugosa* hip, we suspect it was obtained from a paper by Olof E. Stamberg.[4] In this work hips from rose plots in the locality of Moscow, Idaho, were studied by Stamberg. These particular hips belonged to two species, *R. spaldingu* and *R. megalantha*, respectively, and they contained, per 100 gm. of fresh ripe pulp (meat), an average of 1,498 and 1,105 mg. of ascorbic acid, respectively. The meat and seed in these rose hips ranged from 62.8 to 75.0 percent and 25.0 to 37.2 percent, respectively. In the introduction to his paper Stamberg reviewed the literature on rose hips and quoted figures from a paper by Vadova, Beider, and Yanishevskaya[5] in which the Russian investigators found that *R. ciunamomea* and *R. rugosa* ripened early and ranked high in vitamin C potency of from 2,275 to 6,977 mg. per 100 gm. However, Stamberg does not state whether the data are expressed on dry or wet, pulp or whole-fruit basis. He indicates he obtained this information through *Chemical Abstracts* and not from the original publication.

[1] Ledin, Bruce R., The Barbados or West Indian Cherry, Bul. 594, Contrib. Sub-Tropical Experiment Station, Homestead, Fla., 1958.

[2] Asenjo, C. F. and Freire de Guzmán, A. R., The high ascorbic acid content of the West Indian cherry, *Sci.*, **103** 219, 1946.

[3] Mustard, M. J., The ascorbic acid content of some *Malpighia* fruits and jellies, *Sci.* **104** 230–1, 1946.

[4] Stamberg, O. F., Vitamin C and carotene in rose hips and products, *Food Res.*, **10** 392–6, 1945.

[5] Vadova, V. A., Beider, T. I. and Yanishevskaya, M. V., II Biochemistry of the vitamins: Dynamics of the accumulation of vitamin C in dogrose hips, *Proc. Sci. Inst. of Vitamin Research* (USSR) **3** (1) 157–63, 1941.

Recently we have secured through the Kresge-Hooker Scientific Library Translating Service the original publication of Vadova and coworkers.[5] It is clearly stated in this paper that the values reported for *Rosa rugosa* and all the other *Rosa* species investigated are for ascorbic acid in milligrams in 100 gm. of dry fruit pulp. The particular maximum value of 6,977 mg. per 100 gm. of dry pulp quoted by Ledin is for fruits containing 22.0 percent of dry matter in the fruit pulp. Therefore, if this value is calculated on a wet basis, the ascorbic acid content is reduced to 1,535 mg. of ascorbic acid per 100 gm. of wet pulp. In other words, the ascorbic acid content of this highly ranking rose hip, when calculated on a wet basis, is well below the maximum value found for the acerola juice on a wet basis. The semiripe acerola fruit contains in the neighborhood of 80 percent of juice. It should be mentioned here also that dry acerola-juice powder, now locally manufactured in Puerto Rico, ranges between 25,760 to 32,450, and averages 27,220 mg. of ascorbic acid per 100 gm. of powder. These figures have been supplied to us by the laboratory of the Department of Agriculture of Puerto Rico which regularly analyzes samples collected by their inspectors at the manufacturing plant, in Toa Baja, Puerto Rico. Therefore, either on wet or dry basis, the acerola juice ranks higher in ascorbic acid than the pulp of the *R. rugosa* hip.

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(*Continued from inside front cover*)

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[1] As of June 30, 1959.
[2] Leave to pursue studies.
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